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Distribution and trophic structure of benthic communities in a Patagonian fjord (Puyuhuapi Fjord, Southern Chile, 45°S).

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I. General Introduction

1. Main oceanographic characteristics of Chilean fjords

The Chilean Patagonia (41°5'-55°S) is one of the most extensive fjord systems in the world; characterized by a complex geography composed of 3300 islands, peninsulas, channels, straits and fjords, cover an area of ca. 240000 km^2 (Silva & Vargas, 2014). This ecosystem is subject to strong seasonal and latitudinal patterns in precipitation, freshwater discharge, glacier coverage, and light regime (Aracena et al., 2011). The upper layer of the water column is characterized by freshwater discharges from continental runoff, rivers, and glacial tributaries with high silicic acid concentrations (Silva & Calvete, 2002; Silva, 2008). Below the pycnocline, oceanic subantarctic waters (SAAW) increase nutrient concentrations (nitrate and phosphate), resulting in strong vertical and horizontal hydrographic gradients that fluctuate depending on the volume and dominant sources of freshwater input. Temporal and spatial fluctuations of these inflows are related to river discharges, surface runoff fed by high rainfall (1,000-7,000 mm $year^{-1}$; http://www.dga.cl), snow inputs and melting of glaciers.

The interaction between continental and marine inland waters (lateral coupling) are the main drivers of the circulation, hydrographic structure, and productivity patterns throughout the fjord systems (e.g. Acha et al., 2004; González et al., 2010, 2013; Aracena et al., 2011; Jacob et al., 2014; Meerhoff et al., 2014; Paredes et al., 2014; Pérez-Santos et al., 2014; Ross et al., 2014; Torres et al., 2014) since their coupling regulates the flows of nutrients, minerals and organic matter to the adjacent marine ecosystem.

In the inland waters, latitudinal patterns have been described for phytoplankton structure and primary planktonic production (PP), with a decrease in PP towards the South, that would be associated with changes in salinity, optical properties of the water column, and terrestrial flows of silicic acid, especially in areas influenced by proglacial rivers (Aracena et al., 2011; Jacob et al., 2014; Rebolledo et al, 2019). However, large-scale latitudinal patterns forced by geology (active volcanoes) and climate (i.e., temperatures and rainfall) also participate in controlling the productivity and composition of the phytoplankton structure (Torres et al., 2014).

Spatial variations in the production of pelagic food sources (phytoplankton) are reflected in the sediments due to the strong bentho-pelagic coupling that characterize these ecosystems. In agreement with a decrease PP towards the South, Silva & Prego (2002)

observed the same pattern towards the South in the organic carbon content of superficial sediments of fjords and austral channels. Combine with an in situ organic matter production, there is a high contribution of allochthonous organic matter (MOA, terrestrial origin) in marine sediments associated with local coastal runoff and river discharges, these high MOA contents in the head of the fjords decrease towards their mouths (Sepulveda et al., 2011; Silva et al., 2011; Vargas et al., 2011; Quiroga et al., 2012, 2016; Lafon et al., 2014).

2. Sources of organic matter for benthic food webs in Chilean fjords

Fjords are systems of high biological productivity and this ecosystem efficiently export organic carbon to the sediments thus contributing to atmospheric CO_2 sink (Sepulveda et al., 2011; Torres et al., 2014; Bianchi et al., 2020). The carbon sequestration efficiency is influenced by local primary productivity dynamics and/or terrestrial organic matter (tOM) assimilation (Bianchi et al., 2020).

The main source of carbon in benthic food webs correspond to in situ primary planktonic production (Mayr et al., 2011; Sepulveda et al., 2011; Zapata-Hernández et al., 2016). In general, the primary productivity cycle (phytoplankton) of Patagonian fjords (41-51ºS) has been described as a two-phase system consisting of a short non-productive winter phase and a productive phase extending from late winter to autumn (Iriarte et al., 2007; Montero et al., 2011, 2017a). However more recent report describing the occurrence of highly productive blooms of phytoplankton in Patagonian fjords during winter is leading to a reappraisal of the factors that trigger phytoplankton blooms in patagonian fjords (Montero et al., 2017b). Another component of primary productivity are marine macroalgae, an important source of organic matter in coastal systems, contributing to the diet of grazers, suspension feeders and detritivores benthic fauna (Andrade et al., 2016; Zapata-Hernández et al., 2016; Cari et al., 2020).

In addition, allochthonous sources associated with organic matter of terrestrial origin (tOM) represents an important trophic subsidy for planktonic and benthic communities in Chilean fjords (Silva et al 2011; Vargas et al., 2011; Lafon et al., 2014; Zapata-Hernández et al., 2014, 2016; Quiroga et al., 2012, 2016; Cari et al., 2020; Bernal et al., 2020). This tOM made up of leaves and plants is derived from vast forests dominated by the Nothofagaceae family (Rodríguez et al., 2008). This tOM is transported mainly by rivers

into the fjords, generating strong gradients between open ocean areas with less content of tOM in surface sediments toward heads of fjords and close areas to river outlets (Sepulveda et al., 2011; Silva et al., 2011; Rebolledo et al., 2019). Various studies have characterized the origin of organic matter using stable isotopes, stoichiometric relationships of nutrients and lipid biomarkers. These studies have established that a high percentage of organic carbon both in surface sediments and surface layer of the water column is tOM (e.g. Aracena et al., 2011; Vargas et al., 2011; González et al., 2013; Quiroga et al., 2016; Rebolledo et al., 2019).

Another important source of allochtonous OM and inorganic nutrient input into fjord ecosystems come from intensive aquaculture. In many fjords and channels of Chilean Patagonia, intensive aquaculture, mainly salmon farming, has been carried out for more than 30 years. This activity had been associated to the release of large inputs of dissolved and particulate organic, resulting in large contributions of allochthonous P, C and N to fjords (Buschmann et al., 2006; Mayr et al., 2011, 2014; Quiñoñes et al., 2019). Mayr et al (2014) describe for the Comau fjord (42°S) high nutrient supply by the aquaculture farms with a peak of carbon and nitrogen accumulation rates in sediments from 1990s, that according to Häusserman et al (2013) have had negative effects (i.e. biomass and biodiversity decline) on Comau´s fjord epibenthic megafauna. High rates of nutrient discharges (i.e. 12300 t N and 1600 t P annuals) associated with the salmon farming operation have been estimated for the fjord region (Niklitschek et al., 2013). However, the effects of these allochthonous inputs, up to date, not have been evaluated on spatial (e.g. head to mouth fjord) and temporal scales, and had not incorporate the possible effects on food webs (pelagic and benthic). Instead, most of the the evaluations had been carried out in the seafloor directly under salmon cages (Soto and Norambuena 2004; Buschmann 2006; Quiñoñes et al., 2019).

3. Distribution patterns of benthic communities in Chilean fjords

Chilean Patagonia is characterized by intricate channel and island systems, which form a complex environment that resulted in a high diversity of benthic habitats (Häussermann and Försterra, 2009). These fjords present a heterogeneous seafloor, product of glacial retrieval and recent local sedimentary processes; deep walls dominate in some areas as a projection of steep mountains, fine sand and mud with high organic matter content

dominate at river mouth. In addition, a combination of hard substrate with different degree of inclination, soft-bottoms areas and estuarine zones creates a mosaic of environments that had resulted in a high degree of endemism and high benthic biodiversity (Häussermann and Försterra, 2009; Försterra et al., 2016; Betti et al. 2017; 2021; Cardenas and Montiel, 2017; Villalobos et al., 2021).

In this region, benthic communities are commonly dominated by echinoderms (sea stars, brittle stars, sea urchins, sea cucumbers), mollusks, corals, sponges, crustaceans, bryozoans, polychaetes and many other invertebrates. Despite, the rich benthic biodiversity, data on the distribution of benthic communities is generally scarce, and in many cases, only qualitative data is available (Häussermann and Försterra, 2009). In many fjords it is common to find habitat-forming organisms, e.g., *Desmophyllum dianthus* (and other hard coral facies), gorgonian forests, mussel belts, holothurian gardens, *Austromegabalanus psittacus* (and other cirripede aggregations), and different sponges (Häussermann and Försterra, 2009; Betti et al., 2021). These organisms increase available substratum surface areas, offering new habitats for many sessile and mobile benthic organisms that mostly make use of a filter-feeding trophic strategy (Häussermann and Försterra, 2009; Försterra et al., 2016; Cardenas and Montiel, 2017; Betti et al., 2017; 2021). Because they mobilize an important portion of the fresh OM derived from primary production, these organisms dominate and are key to epibenthic communities (Häussermann and Försterra, 2009; Betti et al., 2017; 2021). Distribution patterns of benthic communities followed environmental gradients, bathymetric distribution showing dominance of barnacles and mytilids in upper zone strongly influenced by fresh water and tidal cycles, forming belts and thick banks of these suspension feeders (Häusserman and Försterra, 2009; Betti et al., 2021). In deeper areas (between 70-250 m) it is common to find coral banks and sea-cucumber, polychaetes and anemones aggregations (Häusserman and Försterra, 2009). Cardenas & Montiel (2017) described a well-defined latitudinal gradient, where very abundant gregarious animal (e.g. mollusks bivalves) on the shallow rocky substrate in the sub-Antarctic region tend to decrease in their abundance and distribution towards the Antarctic peninsula. This pattern has been related to the influence of low temperatures, glaciological history and land-ocean interactions (i.e. river discharges). The distribution pattern of the benthic fauna closely related to the temporal and spatial variability of freshwater dynamic from rivers and glaciers (Quiroga et al., 2016; Betti et al., 2017, 2021; Cari et al., 2020). In this context

understanding OM exchange is key to more detailed predictions about secondary production, diversity patterns, and food web dynamics.

4. Trophic ecology

Characterizing food web relationships, including sources and sinks of organic matter, is crucial to understanding trophic ecology and ecosystem dynamics (Pimm et al., 1991; Sokolowski et al., 2012). Tracing sources of primary productivity supporting food webs, and the energy fluxes along them, are difficult tasks. Even when classical approaches (e.g. stomach content analysis) can be applied, these may have several limitations associated with ontogenetic diet switches, different rates of digestion for different preys, unidentifiable gut contents, regurgitation, etc. (Hyslop, 1980).

In the last years, tracing sources of OM consumption and the relative trophic level of organisms in marine food webs have been advanced using isotopic measurements of naturally occurring elements, mainly carbon ($\delta^{13}C/\delta^{12}C$) and nitrogen ($\delta^{15}N/\delta^{14}N$) (Michener & Schell, 1994; Fry, 2006). Assessment of trophic level is based in the principle that stable isotopes usually undergo predictable enrichments between trophic levels (Fry, 1988; 2006). While the relative abundance of ^{13}C is generally enriched less than 1 ‰ between the food and the consumer, ^{15}N undergoes an enrichment of 3-4 ‰ (Fry, 1988; Michener & Schell, 1994). Thus, when two or more isotopically distinct sources of food are present, $\delta^{13}C$ is an excellent indicator of food origin, while $\delta^{15}N$ best indicates trophic level. If food sources have distinct isotopic signatures, the combined use of both isotopes, together with more conventional dietary approaches, can thus be used to provide valuable information on trophodynamics and feeding ecology, as well as spatial use of habitat (Hobson et al., 2002; Post, 2002). Stable isotope analysis is considered a powerful tool to study trophic interactions, and organismal isotopic composition closely reflects the isotopic composition of the diet, plus a predictable isotopic enrichment (Post, 2002; Layman et al., 2007). The isotopic value of an animal's tissue may change over, probably related to a switch to a new diet that is isotopically distinct from the previous diet, or a change in the proportional contributions of dietary sources consumed, each source with a distinct isotopic signature (Michel et al., 2016). Stable isotopes analysis can be used to define trophic niches at the species and community level, as well as identify the isotopic niche space represents the relative position of individuals, populations, and communities in bivariate isotope space, e.g. $\delta^{13}C$ versus

$\delta^{15}N$ (Jackson et al., 2011, Layman et al., 2012), which can yield insights into food resource use. In fact, examining overlap in isotopic niches can help understand niche specialization.

In general, studies addressing benthic trophic ecology in Chilean fjords, including intertidal and well-stratified glacial environments are limited (Mayr et al., 2011; Andrade et al., 2016; Quiroga et al. 2016; Zapata-Hernández et al. 2014, 2016; Cari et al., 2020). The work undertaken so far indicates that isotopic niche width of benthic communities is highly variable in response to environmental conditions and food supply under certain specific environmental conditions such as natural physical disturbances from glacial sedimentation, and a high trophic redundancy (e.g. species with overlapping trophic niches) has been described in fjord environments (Quiroga et al., 2016; Cari et al., 2020).

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II. **Objective and Scientific question of the thesis**

The main objectives of the thesis were (i) to describe distribution patterns and the trophic structure of subtidal benthic communities and (ii) to determine the primary food sources that support these communities along the *Puyuhuapi* fjord.

For this purpose, this thesis was focused on the following questions:

1. How is the distribution of benthic communities along estuarine gradients, in terms of faunal composition and trophic guilds in the *Puyuhuapi* fjord? (Chapter 1)

This topic was evaluated through four sampling campaigns that were carried out during 2019 and 2020 in 12 stations along *Puyuhuapi* fjord, including stations with more oceanic influence adjacent to the *Moraleda* channel. The results obtained allowed us to identify different habitat types (hard and soft bottoms) and high values of species richness, as well as identified the main environmental forces that define the faunal composition and functional traits of benthic communities.

2. Is the trophic structure of benthic communities in the *Puyuhupi* fjord sustained by sources of autochthonous and allochthonous organic matter?

This topic was evaluated through five sampling campaigns that were carried out during different study periods (2018-2020) in 13 stations along *Puyuhuapi* fjord. The data collected was evaluated through stable isotopes analyses of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) using Bayesian mixing model. The results obtained showed an isotopic niche width to be highly variable for benthic communities, probably in response to food supply derived from both autochthonous (marine OM) and allochtonous OM (both from terrestrial organic matter and salmon farming waste).

3. What kind of ecological responses are exhibited by benthic communities under environmental stress (glacier melt and river discharge) in Chilean fjords?

In this topic, we analyzed soft-bottoms benthic communities distribution derived from CIMAR program expeditions (Chile) and isotopic composition of benthic fauna in order to assessment the influence the quality and quantity of food sources for benthic communities in Patagonian fjords. Our results indicate that faunal structure correlates to the distribution of tOM in surface sediments, which gradually decrease towards the outer fjord. There is furthermore a C/N ratio decoupling in benthic fauna, suggesting preferential organic carbon use under limited nitrogen in fjord sediments.

4. What is the influence of allochthonous OM in the faunal composition and trophic structure in epibenthic communities in the *Puyuhuapi* fjord?

In this topic, results from previous chapters were analysed in order to build a synthesis of the benthic communities' structure in a well-stratified fjord with different food sources contributions (i.e. autochthonous and allochthonous). The results indicated the importance of allochthonous OM in trophic ecology and constitute a new approach in which faunal distribution and trophic relationships of benthic communities are integrated to assess the influence of salmon farming activity.

III. List of manuscripts included in this Thesis

PAPER I: Paula Ortiz, Eduardo Quiroga, Paulina Montero, Madeleine Hamame, Federico Betti. "Trophic structure of benthic communities in a Chilean fjord (45°S) influenced by salmon aquaculture: insights from stable isotopic signatures". Marine Pollution Bulletin, Volume 173, Part B, 2021. https://doi.org/10.1016/j.marpolbul.2021.113149.

PAPER II: Eduardo Quiroga, **Paula Ortiz,** Eulogio Soto, Nicole Salinas, Nicole Olguín, Chester Sands. "Geographic patterns of soft-bottoms benthic communities in Chilean Patagonian fjords (47°S-54°S) - influence of environment stress on diversity and stable isotope signatures". Manuscript submitted to Progress in Oceanography (under revision).

IV. List of other publications

During the period of my PhD work I have also contributed to the following publications:

1. Federico Betti, Francesco Enrichetti, Giorgio Bavestrello, Andrea Costa, Alessandra Moreni, Marzia Bo, **Paula Ortiz Saini**, Giovanni Daneri. "Hard-bottom megabenthic communities of a Chilean fjord system: sentinels for climate change? https://doi.org/10.3389/fmars.2021.635430.

2. Madeleine Hamame and **Paula Ortiz**. "Chilean Management areas: assessment of exploitation intensity of commercial species and associated benthic communities, North Patagonia". Manuscript submitted in Frontiers in Marine Science (under revision).

Chapter 1

Benthic habitat characterization of Puyuhuapi fjord (45°S): Integrating hard and soft bottoms benthic communities

Paula Ortiz

Co-authors; Madeleine Hamamé and Eduardo Quiroga

Introduction

Fjords are mainly distributed in the mid-high latitudes, considered Aquatic Critical Zones, because they are sensitive to biogeochemical imbalances related to organic carbon degradation, sequestration and budgets. Which can be affected by anthropogenic pressures and climatic fluctuation (Bianchi et al., 2020). Chilean Patagonian fjords hydrography is highly influenced by environmental conditions associated to different degrees of river runoff. The setting of salinity and organic matter (OM) gradients along the fjord create particular environments that favors the development of a highly diverse benthic community.

The *Puyuhuapi* fjord (44°19'–44°57'S), along its ca. 90 km present a heterogeneous substrate product of erosive glacial action associated to ongoing local sedimentary processes set by retreating ice sheets. Deep walls dominate in some areas as a projection of steep mountains and non-cohesive bottoms result from the contribution of sedimentary material being transported by rivers (Betti et al. 2017; 2021;). Betti *et al* (2021). Steep walls complex epibenthic communities and soft bottom macrobenthos associated to diverse environmental conditions have been described by Betti et al., 2017; 2021 and Häussermann and Försterra, 2009. Soft bottom macrobenthos is mainly dominated by invertebrate species belonging mainly to Polychaeta, Crustacea and Mollusca. In biogeographic terms, most of species described have a wide geographic distribution, some of them showing a strong affinity with the Antarctic and sub-Antarctic region (Cañete et al., 1999; Arntz et al., 2005; Montiel et al., 2005).

Along the *Puyuhuapi* fjord the main anthropogenic pressure is salmon farming, since 1980 their production has expanded intensively. Currently nearly 50% of cultivated salmon is being harvested in the *Aysen* region (43°38'-49°16'S) (Toledo 2016; Nahuelhual *et al*. 2017). A negative impact on benthic communities (mainly gorgonians, anemones and cold-water corals) as a product of anthropogenic activities due to benthic fisheries and salmon farming have been reported for Patagonian fjords (Häussermann et al. 2013; Sanchis et al., 2021). Changes in the sediment load and nutrient inputs negatively influence environmental conditions. It is known that the diversity of benthic communities is highly sensitive to organic enrichment; under conditions of increased nutrient and OM load opportunistic and pollution-tolerant species tend to thrive lowering the diversity of benthic assemblages (e.g., Brown et al., 1987; Gowen and Bradbury, 1987; Weston, 1990; Wildish and Pohle, 2005; Keeley et al., 2019; Tičina et al., 2020; Betti et al., 2021; Sanchis et al., 2021). However, to date, few studies have evaluated the effects of salmon farming on trophic structure or composition (Buschmann et al., 2009; Lojen et al., 2005; Dolenec et al., 2007; Callier et al., 2013; Woodcock et al., 2018; Sanchis et al., 2021). Because of the potential negative impact of salmon farming it is urgent to establish ecological and environmental baselines that could in the future allow to identifying shifts in community structure and functioning in fjord ecosystems. Therefore, this chapter aim is to describe the hard and soft bottoms benthic communities in the *Puyuhuapi* fjord and adjacent areas, by classifying the benthic environment, the taxonomic and functional groups (trophic classification).

Materials & Methods

Study area

The *Puyuhuapi* fjord, in the south of Chile (Northern Patagonia, 45°S), is ~90 km long, ~7 km wide, and average depth 250 m. At its southern section, the mouth of the fjord is connected to the *Moraleda* channel; and, in its middle, to the *Jacaf* channel (Fig. 1). The hydrography is characterized by an estuarine circulation with a vertical two-layer structure comprising a surface (5-10 m) freshwater layer highly variable, more oxygenated, less saline and with lower nutrient concentrations of nitrate and orthophosphate, compared to the subpycnocline layer (Schneider et al., 2014; Montero et al., 2017; Perez-Santos et al., 2018). The upper layer receives allochthonous organic matter inputs from mainly river discharge and rainfall (up to 3000 mm y^{-1}) (DGA, 2003).

The Cisnes River is one the most important freshwaters input in the study area contributes an average flow of 233 m^3s^{-1} (Prado-Fiedler and Castro, 2008). Main anthropogenic activities in the fjord are intensive salmon farming: of the total 723 farm permits in the *Aysén* region (https://mapas.subpesca.cl/ideviewer/), 86 are localized in the study area.

Survey methods

Four campaigns were performed between March 2019 and December 2020. A total of 12 stations (Fig. 1; Table 1) were selected along the fjord (middle, mouth and outer sections), considering 0- and 90-meters depth. The area sampled covered soft, mixed and hard substrates. Epibenthic communities were studied using a Remote Operated Vehicle (ROV; model MS1-Mariscope) equipped with an HD/4K camera mounted at an oblique angle to the seafloor, and sensors monitoring position (compass) and depth. In each station, 1-4 transects were recorded, the record time varied between 4 to 30 minutes per transect. The transect orientation were defined according to the inclination of substrate (i.e. horizontal, sloping or vertical).

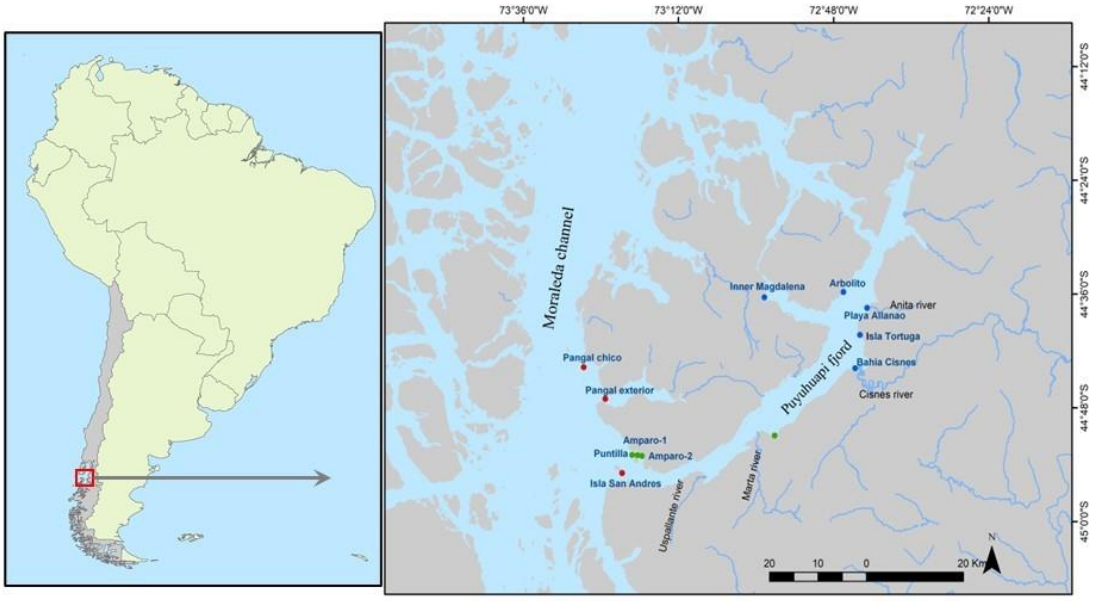

Figure 1. Study stations along the *Puyuhuapi* fjord. The color of the circles represents the different sections; blue= middle; green= mouth and red= outer.

In each station taxa/species were registered, generating a matrix of presence and absence data. The fauna was identified using specialized literature (Forcelli, 2000; Häussermann

and Försterra, 2009; Reyes and Hüne, 2012; Betti et al, 2017). For some taxa identification was only possible at higher taxonomic levels. Functional groups were assigned based on the feeding guild for each taxa/species using literature (Häussermann and Försterra, 2009; Reyes and Hüne, 2012; Betti et al, 2017). The date, time, geographical position, depth and substrate inclination were recorded in each transect (see Supplementary data).

Soft-bottom macroinfaunal communities were sampled in four stations using a modified van Veen grab (0.05 m^2). In each station, 3-4 independent replicates of sediment were sampled (Table 1). Sediment samples were sieved through a 500-μm mesh size screen and biological material was fixed using buffered 4% formaldehyde solution in seawater. In the laboratory, the macroinfauna was sorted and identified using a stereomicroscope. Feeding guilds for polychaetes families was classified using Fauchald and Jumars (1979) and Jumars et al. (2015); and other macroinfauna taxa using Häussermann and Försterra (2009). The functional traits assigned of macroinfaunal taxa/species were divided into five feeding modes: carnivores (CA), omnivores (OM), surface deposit feeders (SDF), sub-surface deposit feeders (SSDF) and suspension-filter feeders (SS). Density data of each replicate was standardized by area (m^2) and used to determine the mean and standard deviation at each station. The sediment parameters such as (1) Total Organic Matter Content was determined by the calcination method (Luczack et al., 1997), and (2) pH/ORP as described by Hargrave et al. (2008).

Data Analyses

Epibenthic community assemblage were analyzed by station using multivariate and univariate methods. A hierarchical cluster analysis was applied to search associations between the taxa/species and distribution of the stations along estuarine gradient. Presence/absence values were used to performed resemblance measure based on a Bray Curtis similarity matrix. Clustering was performed using the SIMPROF routine, with 1000 mean permutations and 999 simulation permutations, and using a significance level of 5%. An analysis of similarity test (one-way ANOSIM) was used to evaluate similarities between stations along the fjord. The cluster analysis and ANOSIM test were performed with the software PRIMER V6.1.6 (Clarke et al., 2008).

To identify key environmental variables, a generalized lineal model (GLM) was used. Environmental variable considered in this analysis were depth, section fjord (middle=1, mouth=2 and outer=3), substrate inclination (vertical, sloping, horizontal and sloping/horizontal) and substrate type (hard/mixed, soft/mixed and hard bottoms). Since independent variable considered only presence/absence of species, binomial distribution was used. Only taxa with higher presence (≥ 25% of the stations) and only sections with similar substrate characteristics (section 2 and 3) were considered in this analysis. The analyses were performed in R studio. Kruskal-Wallis nonparametric with post-hoc Dunn tests were used to assessment differences in the species richness, trophic groups and abundances among stations for the benthic communities. All analyses were performed using software Statistica 7.0.

Table 1. Summary of sampling campaigns in the *Puyuhuapi* fjord (ROV and sedimentary samples), indicating the geographical coordinates, date, fjord section, substrate type, sampling gear and depth for each sampling station.

Station	Latitude (°S)	Longitude (°W)	Date	Fjord Section	Substrate type	Sampling gear/Depth (m)
Pangal Exterior	44°48'29.988"	73°22'13.08"	07-03-2019	Outer	Mixed	ROV/60-92
Isla San Andres	44°56'17.556"	73°19'21.215"	07-03-2019	Outer	Hard and mixed	ROV/0-27
Pangal Chico	44°45'11.83"	73°25'41.34"	08-03-2019	Outer	Hard and mixed	ROV/36-50
Isla Tortuga	44°41'5.03"	72°42'36.56"	17-01-2020	Middle	Soft	ROV/0-8 and Grab/25-30
Playa Allanao	44°38'12.1"	72°41'38.7"	17-01-2020	Middle	Soft	ROV/0-40
Arbolito	44°36'35.7"	72°45'24.56"	18-01-2020	Middle	Mixed	ROV/4-9 and Grab/17
Puntilla	44°54'21.7"	73°17'52.3"	08-03-2020	Mouth	Hard	ROV/0-35
Amparo-1	44°54'26.2"	73°16'18.35"	10-03-2020	Mouth	Hard and mixed	ROV/0-44
Amparo-2	44°54'51"	73°17'0.75"	11-03-2020	Mouth	Hard	ROV/0-15
Río Marta	44°51'54.17"	72°55'55.25"	12-03-2020	Mouth	Hard and mixed	ROV/4-70 and Grab/34
Bahia Cisnes	44°44'36.25"	72°43'16.25"	11-12-2020	Middle	Soft	Grab/30
Inner Seno Magdalena	44°37'22.7"	72°57'41.91"	12-12-2020	Middle	Mixed	ROV/20-50 and Grab/21

Results

Environmental characterization

The study area was divided in three sections (middle, mouth and outer) based on an estuarine gradient, influenced by a strong horizontal stratification (head to mouth of fjord) with a vertical two-layer structure comprising a surface (5-10 m) freshwater layer seasonally variable and a more stable subeuphotic layer associated to Sub-Antarctic waters (Schneider et al., 2014; Montero et al., 2017; Perez-Santos et al., 2018). In this fjord, a high contribution of terrestrial organic matter (tOM) in surface sediments and water columns have been reported (Silva et al., 2011; Sepulveda et al., 2011). The bathymetry of *Puyuhuapi* fjord is complex, with variable bottom depths that could reach more than 250 m, the fjord has a sill located in the southern section around 170 m depth (Schneider et al 2014). Hypoxic conditions in the water column have been detected below 100 m depth, where oxygen concentrations have been found to be as low as <2 ml L^{-1} (Schneider et al., 2014; Pérez-Santos, 2018). Information on submarine morphology and bathylithological characterization in Chilean fjords is scarce, but the erosive action of glaciers is recognized, defining a deep and central zone in fjords characterized by presenting fine sediment associated with glacial deposition processes, and a coastline that it can present different surfaces, such as rocky cliffs, rocky outcrops and non-cohesive bottoms as a result of the contribution of sedimentary material mainly from the rivers of the Andean Cordillera, and by non-marine processes such as landslides and moraine deposits (Häussermann and Försterra, 2009).

ROV surveys covered 11 coastline stations along the *Puyuhuapi* fjord and adjacent areas (Fig. 1), with three main types of substrates; hard, mixed and soft bottoms. The mixed bottom corresponded to a combination between hard and soft bottoms. In each of these substrates, differences in their inclination of substrate; as well as substrate composition (presence of boulders, sand, shell cover, organic detritus and coralline algae cover), were visually assessed (see Supplementary data). Generating a complex environment in terms of their habitats.

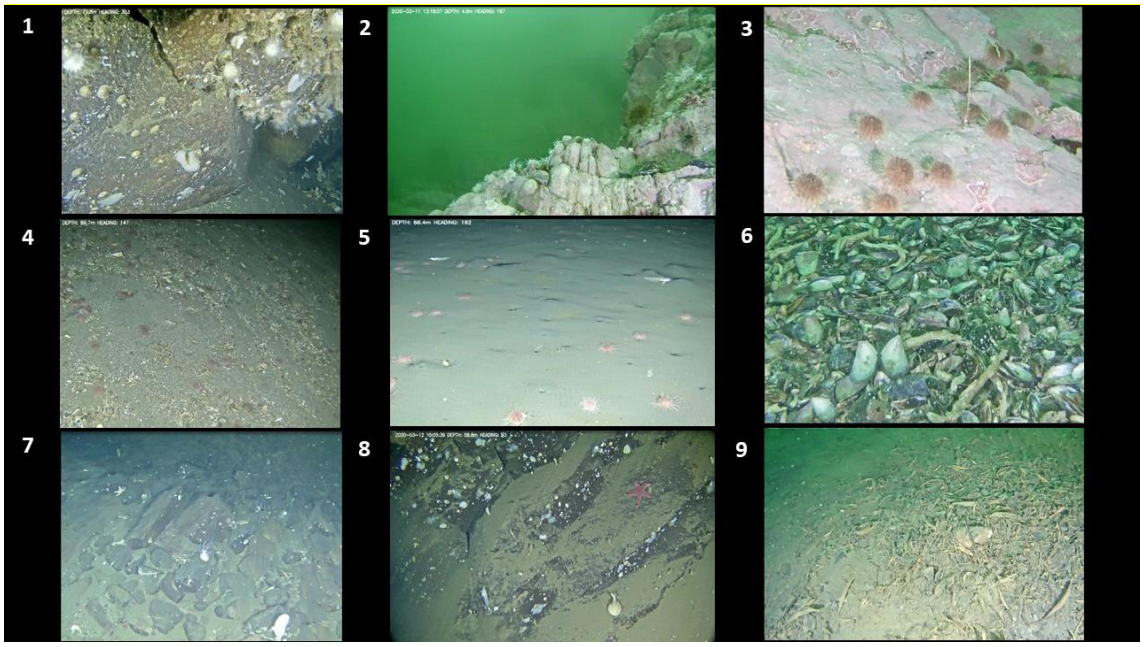

Figure 2. Substrate types identified in *Puyuhuapi* fjord and adjacent areas. 1-2-3 Hard Bottom; 1-2. Steep rocks, 3. Rocks with coralligenous algae. 4 to 7 Non cohesive bottoms; 4. Sand, 5. Mud, 6. Shells cover, 7. Boulders. 8. Detritus, 9. Organic detritus.

Epibenthic communities

Based on analyzed images using ROV in this study, we identified 108 taxa/species in the 11 stations along the *Puyuhuapi* fjord and *Moraleda* Channel (Table 2). In general, the main taxonomic groups were Porifera (25 taxa/species), Echinodermata (19), Cnidaria (15) and Mollusca (13). In contrast, Bryozoa (7), Polychaeta (7), Fishes (7), Algae (6), Crustaceans (5), Ascidians (3) and Brachiopoda (1) exhibited a lower species richness (Table 2).

For section fjord, the species richness of the epibenthic fauna presented low values (≤4 taxa/species) in the stations located in the middle of the *Puyuhuapi* fjord associated to soft and mixed bottoms with high content of organic detritus (Playa Allanao, Isla Tortuga, Arbolito and Inner Seno Magdalena stations), being Arthropoda, Echinodermata and Annelida best represented phyla. The highest values of species richness (between 14 and 38 taxa/species) were recorded in stations located at the mouth and outer *Puyuhuapi* fjord associated mainly with hard bottoms, represented by a greater diversity of phyla (i.e. Porifera, Cnidaria, Mollusca, Echinodermata, Artropoda and Bryozoa) (Fig. 3 and Table 2).

Porifera presented a low taxonomic definition due to the observation method (ROV images). However, the main morphotypes belong to massive and encrusting sponges. It is important to mention that for future studies an improved identification work is necessary due to the importance of this group in the study area (Bertolino et al., 2019; 2020; Betti et al., 2021).

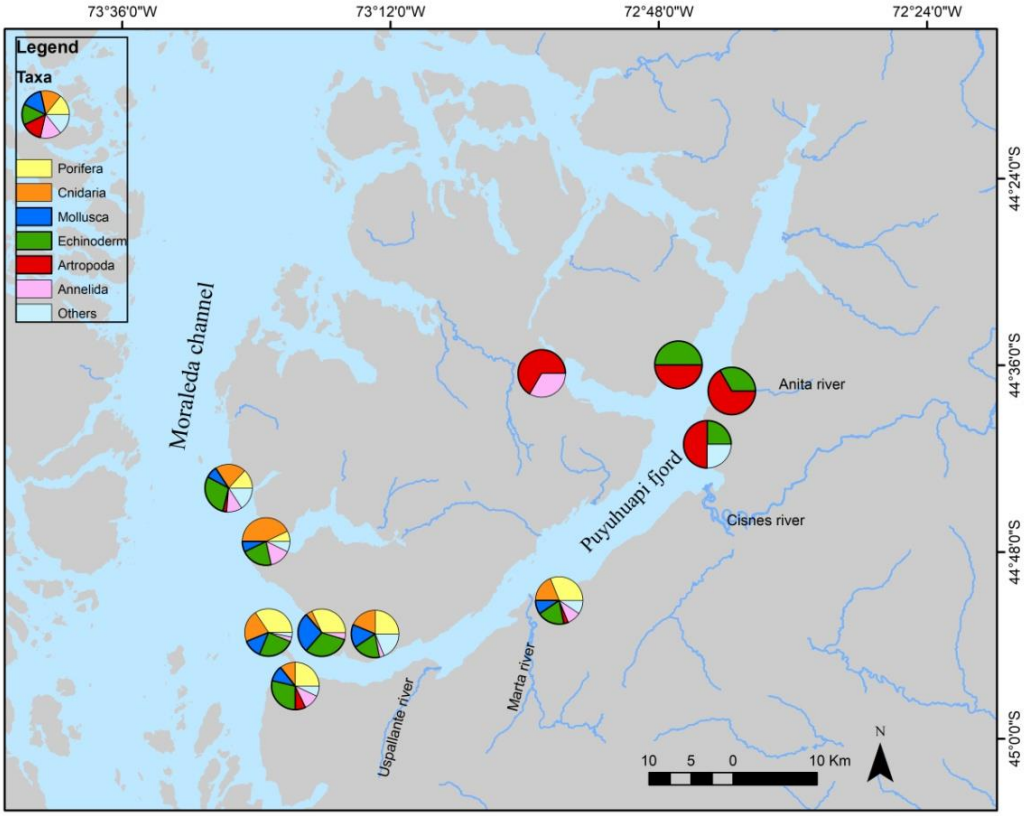

Figure 3. Spatial representation of epibenthic fauna phyla in the *Puyuhuapi* fjord and outer stations. Others: Brachiopoda and Ascidiacea.

Depth and section fjord were used to assessment the differences among taxa and functional groups. Depth was a good predictor of the presence of Porifera unidentified 6, *Metridium senile*, *Cosmasterias lurida*, *Arbacia dufresnii* and *Thouarella* sp. (>80%, GLM, $p<0.05$). Except for *Thouarella* sp., which were associated to shallow waters, above 20 m. *Thouarella* sp. was associated to deep waters, below 25 m (GLM, $p<0.05$). The section of fjord (i.e. estuarine gradient) related presence of Porifera with mouth located stations (56-72%, GLM, $p< 0.05$) and presence of serpulids polychaetes with outer fjord stations (88%, GLM, $p< 0.05$).

Table 2. List of taxa/species recorded with ROV for 11 stations sampled along *Puyuhuapi* fjord and outer stations located in *Moraleda* Channel.

			Outer Puyuhuapi fjord			Mouth Puyuhuapi fjord				Middle Puyuhuapi fjord			
Phylum	Taxa/species	Trophic Group	Pangal Exterior	Isla San Andres	Pangal Chico	Río Marta	Puntilla	Amparo-1	Amparo-2	Isla Tortuga	Playa Allanao	Arbolito	Inner Seno Magdalena
Rodhophyta	Gracilariaceae									X		X	
Rodhophyta	Corallinales 1						X		X				
Rodhophyta	Corallinales 2						X	X	X			X	
Chlorophyta	Cladophoraceae									X	X	X	
Chlorophyta	Ulva sp									X			
Ochrophyta	*Macrocystis pyrifera*			X						X			
Porifera	*Cliona chilensis*	SS		X			X		X				
Porifera	*Axinella crinita*	SS		X	X		X	X	X				
Porifera	*Haliclona* sp.	SS					X						
Porifera	*Tedania* sp.	SS					X						
Porifera	*Tethya* sp.	SS					X						
Porifera	Porifera 1	SS				X	X	X					
Porifera	Porifera 2	SS		X		X	X	X	X				
Porifera	Porifera 3	SS				X							
Porifera	Porifera 4	SS				X	X	X	X				
Porifera	Porifera 5	SS				X							
Porifera	Porifera 6	SS		X			X	X	X				
Porifera	Porifera 7	SS				X							
Porifera	Porifera 8	SS				X							
Porifera	Porifera 9	SS				X							
Porifera	Porifera 10	SS				X							
Porifera	Porifera 11	SS				X	X	X	X				
Porifera	Porifera 12	SS							X				
Porifera	Porifera 13	SS						X					
Porifera	Porifera 14	SS					X	X					
Porifera	Porifera 15	SS	X		X								
Porifera	Porifera 16	SS		X									
Porifera	Porifera 17	SS		X									
Porifera	Porifera 18	SS			X								
Porifera	Porifera 19	SS		X	X								
Porifera	Porifera 20	SS			X								
Cnidaria	*Metridium senile*	CA		X			X	X					
Cnidaria	*Anthotoe chilensis*	CA			X		X		X				
Cnidaria	*Bolocera* sp.	CA	X	X									
Cnidaria	*Actinostola chilensis*	CA	X		X								
Cnidaria	*Mesozoanthus fossii*	CA	X										
Cnidaria	*Dactylanthus antarcticus*	CA			X								
Cnidaria	Actinaria	--			X								
Cnidaria	*Primnoella chilensis*	SS	X	X	X	X	X	X					
Cnidaria	*Thouarella* sp.	SS	X		X	X		X					
Cnidaria	*Clavularia* sp.	SS				X	X	X					
Cnidaria	*Alcyonium* sp.	SS			X		X						
Cnidaria	Scleractinia	SS				X	X	X					
Cnidaria	*Desmophyllum dianthus*	SS	X		X								
Cnidaria	Octocorallia	--				X							
Cnidaria	Hydrozoa	--				X	X	X					
Mollusca	*Chiton* sp.	GR					X	X	X				
Mollusca	*Chiton bowenii*	GR		X					X				
Mollusca	*Aulacomya atra*	SS				X	X	X					
Mollusca	*Mytilus chilensis*	SS							X				
Mollusca	*Zygochlamys patagonica*	SS		X	X								
Mollusca	Bivalvia	SS	X	X	X								
Mollusca	*Tegula atra*	GR						X	X				
Mollusca	*Nacella* sp.	GR							X				
Mollusca	*Crepipatella* sp.	GR							X				
Mollusca	*Fusitriton magellanicus*	CA			X								
Mollusca	*Argobuccinum ranelliforme*	CA					X	X					
Mollusca	Gastropoda	--				X							
Mollusca	Nudibranchia	CA				X	X	X					
Annelida	Terebellidae 1	DD	X		X								
Annelida	Terebellidae 2	DD				X							
Annelida	*Chaetopterus variopedatus*	SS			X								
Annelida	Sabellidae	SS		X									
Annelida	Serpulidae	SS	X	X	X	X		X	X				
Annelida	Polychaeta 1	--		X	X								
Annelida	Polychaeta 2	--				X	X						X

Continuation Table 2. List of taxa/species recorded with ROV for 11 stations sampled along *Puyuhuapi* fjord and outer stations located in *Moraleda* Channel.

			Outer Puyuhuapi fjord			Mouth Puyuhuapi fjord				Middle Puyuhuapi fjord			
Phylum	**Taxa/species**	**Trophic Group**	**Pangal Exterior**	**Isla San Andres**	**Pangal Chico**	**Río Marta**	**Puntilla**	**Amparo-1**	**Amparo-2**	**Isla Tortuga**	**Playa Allanao**	**Arbolito**	**Seno Magdalen**
Artrophoda	Cirripedia	SS				X				X	X	X	
Artrophoda	*Munida gregaria*	DD		X									X
Artrophoda	*Paralomis granulosa*	CA		X									
Artrophoda	*Propagurus gaudichaudi*	CA			X								
Artrophoda	*Metacarcinus edwardsii*	CA-DD								X	X	X	X
Brachiopoda	*Magellania venosa*	SS					X	X					
Briozoa	*Cellaria* sp.	SS			X	X							
Briozoa	*Adeonella* sp.	SS			X								
Briozoa	*Aspidostoma giganteum*	SS	X		X	X		X					
Briozoa	*Reteporella magellanica*	SS			X	X		X					
Briozoa	Bryozoa 2	SS						X		X			
Briozoa	Bryozoa 3	SS						X					
Briozoa	Bryozoa 4	SS			X								
Echinodermata	*Cosmasterias lurida*	CA		X	X	X	X	X	X	X	X	X	
Echinodermata	*Glabraster antarctica*	CA		X	X		X		X				
Echinodermata	*Meyenaster gelatinosa*	CA					X						
Echinodermata	*Ganeria falklandica*	CA				X	X						
Echinodermata	*Cycethra verrucosa*	CA			X								
Echinodermata	*Odontaster penicillatus*	CA			X								
Echinodermata	Asteroidea 1	CA					X	X	X				
Echinodermata	Asteroidea 2	CA										X	
Echinodermata	Asteroidea 3	CA				X							
Echinodermata	Asteroidea 4	CA				X							
Echinodermata	Asteroidea 5	CA	X										
Echinodermata	Asteroidea 6	CA		X	X								
Echinodermata	Asteroidea 7	CA		X	X								
Echinodermata	*Arbacia dufresnii*	GR		X	X		X	X	X				
Echinodermata	*Loxechinus albus*	GR	X	X	X		X		X				
Echinodermata	*Heterocucumis godeffroyi*	SS		X	X	X	X	X	X				
Echinodermata	*Psolus* sp	SS	X	X	X			X					
Echinodermata	Holothuroidea	--				X		X	X				
Echinodermata	*Gorgonocephalus chilensi*	SS			X								
Chordata	*Sycozoa* sp.	SS		X									
Chordata	Ascidiacea 1	SS		X	X								
Chordata	Ascidiacea 2	SS						X					
Chordata	*Sebastes oculatus*	CA		X	X	X	X			X		X	
Chordata	*Pinguipes chilensis*	CA		X		X	X	X		X			
Chordata	*Congiopodus peruvianus*	CA									X		
Chordata	*Prolatilus jugularis*	CA	X	X	X								
Chordata	Patagonotothen	CA	X		X								
Chordata	Nothoteniidae	CA				X		X	X		X		X
Chordata	Perciforme	--				X							
	N° Taxa/species		16	32	41	36	36	35	25	10	6	8	4

Trophic composition of the epibenthic fauna was grouped in (1) Suspension feeders (SS: 54 taxa/species), (2) Carnivores (CA: 32 taxa/species), (3) Grazers (GR: 6 taxa/species) and (4) Deposit feeders (DD: 3 taxa/species) (Table 2). The SS group was important in the stations located in the mouth and outer compared to the middle section of the *Puyuhuapi* fjord (Fig. 4). The most taxonomically diverse group were the SS, which included many sponges, as well as octocorals, bivalves, polychaetes, bryozoans, holothuroids, and ascidians. This feeding group presented higher number of taxa at the mouth of the fjord compared to the middle section (Kruskal-Wallis test, $p < 0.05$).

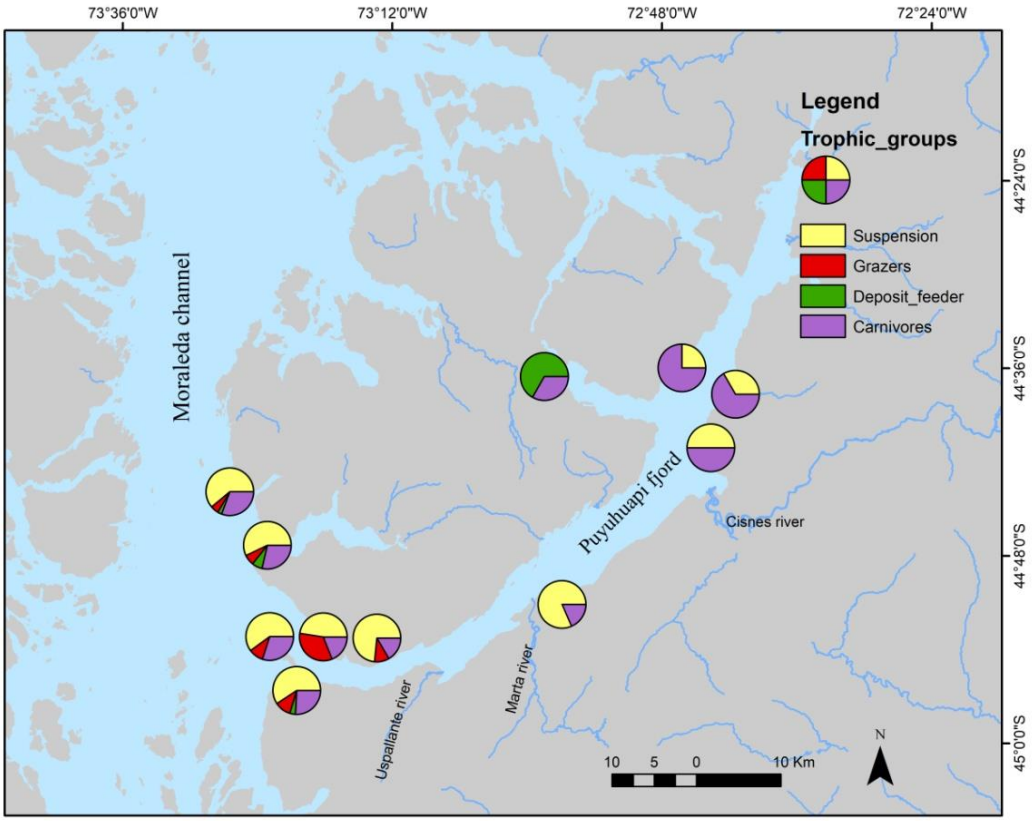

Figure 4. Spatial representation of epibenthic fauna trophic groups in the *Puyuhuapi* fjord and outer stations.

Cluster analysis, based on data of presence and absence, was realized using 95 taxa/species of invertebrates, excluding algae and fishes. Our results showed three groups that according to the fjord section (Fig. 5). A first cluster (Habitat 1) associated with soft-bottoms (mud, sand and mixed) at Arbolito, Isla Tortuga, Playa Allanao and Inner Seno Magdalena stations. All these stations were located in the middle of the *Puyuhuapi* fjord. A second cluster (Habitat 2) was characterized by hard bottoms associated to steep rocks, located in outer fjord and a third cluster (Habitat 3) characterized by hard bottoms associated by rock walls, including mouth (Puntilla, Amparo-1 and Amparo-2) and middle fjord (Río Marta) (Fig. 5). The one-way ANOSIM showed significant differences between groups (Global R =0.90, $p<0.05$).

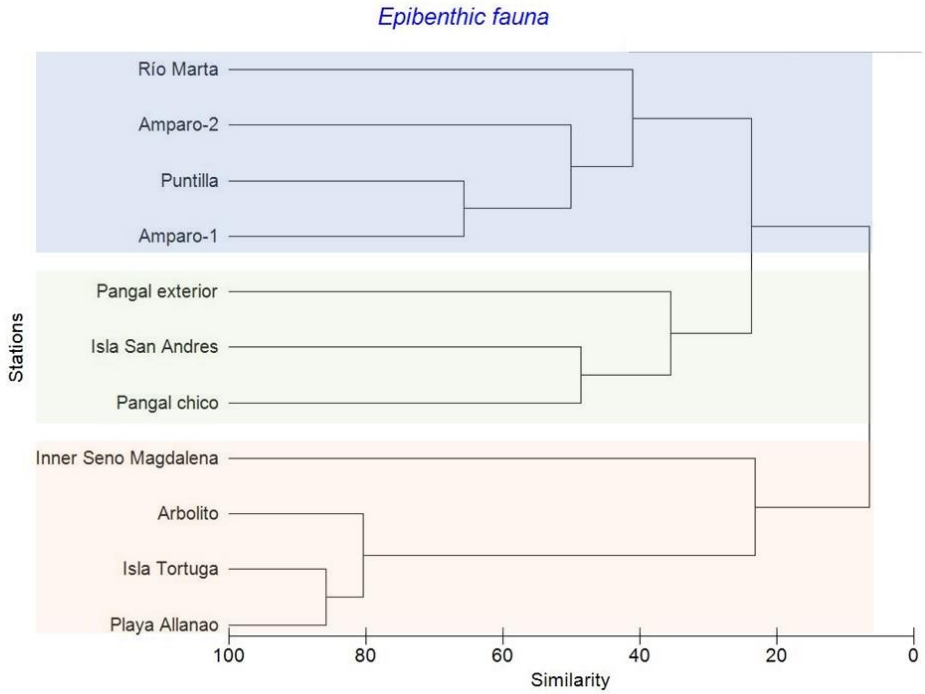

Figure 5. Dendrogram based on presence-absence values of epibenthic fauna from *Puyuhuapi* fjord. Groups were classified using hierarchical cluster analysis using the Bray-Curtis similarity index, and different colors indicate the three main clusters.

Habitat descriptions

Habitat 1: Barnacles, asteroids & crustaceans
Main substrate: Mixed bottom with organic detritus
Substrate inclination: Horizontal
Section: Middle fjord, *Puyuhuapi*
Stations: Sector Arbolito, Isla Tortuga, Playa Allanao and Inner Seno Magdalena
Depth range: 0-50 m

This habitat was associated to different depth ranges (1 to 20 m; 20 to 50 m), with a deeper range only registered in Playa Allanao and Inner Seno Magdalena stations. The epibenthic fauna was associated with mixed sediments (rocks, sand, mud, shell cover and organic detritus). At the Playa Allanao station, bacterial mats on bottoms were identified. Substrate inclination was horizontal, whose benthic assemblage exhibited a low epibenthic species richness (n = 7 taxa/species), characterized by asteroids (2), bryozoa (1), polychaeta (1) and crustacean (3 species) (Supplementary data). Crustaceans was one of the most important groups in terms of frequency in the middle section of the fjord (Kruskal-Wallis test, $p < 0.05$), probably due to presence of cirripedia and *Metacarcinus edwardsii* compared to the other sections.

Munida gregaria appear to be a dominant species in the Inner Seno Magdalena station constituting well-defined aggregations. This decapod has been reported for the study area, in high densities (114 ± 9 ind./m^2), particularly in rivers mouth and shallow areas (Betti et al., 2017; Figueroa-Muñoz et al., 2020). In these areas, particulate organic carbon concentration was high associated with terrestrial organic matter (Sepulveda et al., 2011; Silva et al., 2011), which is an important food source for crustaceans and other detritivore species such as polychaetes and echinoderms (Quiroga et al., 2016; Cari et al., 2020). These species play a key ecological role in energy transfer between different trophic levels (Betti et al., 2020; Bernal et. al., 2020; Ruiz-Ruiz et al., 2021).

On the other hand, scavenging crustaceans constitute an important food source for fishes (e.g. *Eleginops maclovinus*, *Pinguipes chilensis*, *Sebastes oculatus*; Reyes and Hüne, 2012) and dolphins (*Cephalorynchus eutropia* and *Lagenorhynchus australis*) which inhabiting in the study area. In the *Puyuhuapi* fjord, an important source of organic matter and nutrient inputs is provided by salmon farming activity (Buschmann et al., 2006; Quiñones et al., 2019). This organic matter load can generate organic enrichment in the seabed, producing changes in the biogeochemical characteristics of the sediments, as well as in the structure of benthic communities (e.g. Gowen & Bradbury 1987; Brown et al., 1987; Weston, 1990; Dauvin et al., 2020; Sanchis et al., 2021). These communities can remineralizer an important fraction of this organic matter, but if this capacity for assimilating organic matter is exceeded, a hypoxic or anoxic condition can be reached, producing changes in biogeochemical properties of sediment and benthic biota (e.g. Weston, 1990; Borja et al., 2000; Dauvin et al., 2020). These microbial communities have been reported in the *Puyuhuapi* fjord associated with organic waste located under salmon cages, in both soft and hard bottoms (http://www.sernapesca.cl). However, in sites associated to rivers entry i.e. Playa Allanao, bacterial mats have been identified.

Habitat 2: Sponges, anemones, octocorales, holothuroidea aggregations, sea urchin & serpulids polychaetes.
Main substrate: hard bottom with shells and organic detritus cover.
Substrate inclination: Sloping.
Section: *Moraleda* Channel, outer *Puyuhuapi* fjord.
Stations: Pangal Exterior, Pangal Chico and Isla San Andres.
Depth ranges: 0-50 m and 64-92 m.

Habitat-2 was found on hard bottoms composed by rocks covered with coralligenous algae in waters depth above 50 m, and rocks without coralline algae coverage at deep of 50-90 m. Additionally, shells cover and organic detritus were observed in these substrate types (Supplementary data).

Substrate inclination was sloping, whose benthic assemblage exhibited a high species richness (n = 52), dominated by Echinodermata (12), Cnidaria (11), Porifera (10), Polychaeta (5), Bryozoa (5), Mollusca (4), Crustacea (3), Ascidiacea (2) (Table 2). Echinodermata and Cnidaria were recorded in higher densities and species richness; Echinoderms= *Cosmasterias lurida*, *Glabraster antarctica*, *Cycethra verrucosa*, *Odontaster penicillatus, Arbacia dufresnii*, *Loxechinus albus*, *Heterocucumis godeffroyi*, *Psolus* sp. and *Gorgonocephalus chilensis*; and Cnidaria= represented main by anemones (*Metridium senile*, *Anthotoe chilensis*, *Bolocera* sp., *Actinostola chilensis* and *Dactylanthus antarcticus*) and octocorales (*Primnoella chilensis*, *Thouarella* sp., *Alcyonium* sp. and *Desmosphyllum dianthus*) (Supplementary data).

In this habitat, we identified to *Zygochlamys patagonica* and cold waters corals i.e *Desmosphyllium dianthus*, forming banks at 40 m and 70 m depth. *Actinostola chilensis* was also present only in these stations, this species has been described as commonly found in the vicinity of coral banks (Cairns et. al., 2005).

The red sea-urchin (*Loxechinus albus*) dominated covered different substrate types, from mud to rocky bottom and from surface till deep waters up to 90 m. In all stations were registered serpulids polychaetes, this family was better represented in this habitat compared to the other sections of the fjord (GLM, $p < 0.05$)

Habitat 3: Sponges, octocorales and holothuroidea aggregations & sea urchin.
Main substrate: hard bottoms.
Substrate inclination: Vertical and sloping.
Section: Mouth *Puyuhuapi* fjord and one station in Middle of the fjord (Rio Marta).
Stations: Puntilla, Amparo-1, Amparo-2 and Rio Marta.
Depth range: 0-44m.

Habitat-3 was associated to different depth ranges, with a depth range 0-15 m for Amparo-2, but in general depths varied from 0 to 44 m for the other stations. This habitat was found on hard bottom composed of steep walls and rocks steep, with sand and shells

cover. The River Marta station presented lower substrate inclination and higher contribution of organic detritus than the other stations (Supplementary data).

In this habitat the main substrate inclination were vertical and sloping, whose benthic assemblage exhibited a high species richness (n= 61) dominated by Porifera (19), Echinodermata (12), Mollusca (10) and Cnidaria (9) (Table 2). Echinodermata, Cnidaria and Mollusca were important in terms of species richness. Echinoderms such as *Cosmasterias lurida*, *Glabraster antarctica*, *Meyenaster gelatinosa*, *Ganeria falklandica*, *Arbacia dufresnii*, *Loxechinus albus*, *Heterocucumis godeffroyi* and *Psolus* sp were registered. Cnidaria exhibited species such as *Primnoella chilensis*, *Thouarella* sp., *Alcyonium* sp. and *Clavularia* sp (Supplementary data).

A high number of species of mollusks were registered at Puntilla and Amparo-2, compared to the other sections of the fjord (Kruskal-Wallis test, $p<0.05$). In Puntilla, *Argobuccinum ranelliforme*, *Aulacomya atra*, *Chiton* sp. and Nudibranchia were dominant. In contrast, in Amparo-2, *Chiton bowenii*, *Chiton* sp., *Crepipatella* sp., *Mytilus chilensis*, *Nacella* sp and *Tegula atra* were frequent.

In this habitat, the octocoral *Clavularia* sp and the Brachiopoda *Magellania venosa*, were dominants, particularly in depths greater than 20 m. Also, the phylum Porifera was dominant in this habitat.

Soft bottom communities

A total of 43 taxa/species (>0.5 mm) were registered in the middle section of the fjord, polychaetes were the most abundant (Fig. 6), belonging to families Cirratulidae, Capitellidae, Cossuridae, Paraonidae, Spionidae, Sabellidae and Ampharetidae. All these families were characterized by small bodied. Mollusks (7 taxa/species) and crustaceans (6 taxa/species) were also important (Table 3; Fig. 6) as have been reported in other Patagonian fjords (Montiel et al., 2011; Quiroga et al., 2013; Rozbaczylo et al., 2017; Sanchis et al., 2021).

The mean abundances values of macroinfauna varied from 3040 ± 2048 ind/m^2 in Rio Marta to 15055 ± 4782 ind/m^2 in Bahía Cisnes (Table 3). The polychaete families; Capitellidae (3720 ± 1051 ind/m^2), Paraonidae (3640 ± 309 ind/m^2) and Spionidae (2675 ± 2814 ind/m^2) presented significantly higher abundances in Bahía Cisnes than in Rio

Marta station (paired t test; $p<0.05$). In Isla Tortuga, the families Cossuridae (3380 ± 1388 ind/m^2), Sabellidae (1093 ± 550 ind/m^2) and Ampharetidae (2500 ± 1101 ind/m^2) presented significantly higher abundances than in Inner Seno Magdalena (Sabellidae and Ampharetidae) and Rio Marta (Cossuridae) stations (paired t test; $p<0.05$). In Inner Seno Magdalena, Cirratulidae presented significantly higher abundances (1850 ± 231 ind/m^2) than in Isla Tortuga station (paired t test; $p<0.05$).

These results indicate higher mean densities of soft-bottom macroinfauna in the *Puyuhuapi* fjord, compared with abundances estimated for other fjords in the Magellanian region (47°-52°S; range abundances: 627-1857 ind/m^2; Thatje and Mutschke, 1999) and northern Patagonia (39°-44°S; range abundances: 3645-7726 ind/m^2; Jaramillo et al., 1984; Palma et al., 2005). At Bahía Cisnes and Isla Tortuga, abundances were within the same order magnitude (~ 10000 ind/m^2) reported for fjords associated to important rivers contributions such as Baker and Aysen (Quiroga et al., 2012, 2013), and inner marine areas with salmon farming influence (> 10000 ind/m^2) in northern Patagonia (Sanchis et al., 2021).

In terms of feeding modes, deposit feeders (DD: 21 taxa/species) was important group, reaching 80% in stations dominated by polychaete families, except at Rio Marta was represented by omnivores (OM: 6 taxa/species) and carnivores (CA: 7 taxa/species) (Fig. 7). Suspension feeders (SS: 7 taxa/species) were represented by high dominance of Sabellidae (10% in Isla Tortuga) and Ostracoda (11% in Inner Seno Magdalena and 8% in Bahia Cisnes). The abundances of DD and SS feeding modes were significantly higher in Bahia Cisnes than in Rio Marta station (paired t test; $p<0.05$).

Bahía Cisnes presented high abundance of families (Capitellidae and Cirratulidae) considered bioindicators of polluted environments (Bellan, 1980; Sanchis et al., 2021) and high abundances of polychaetes (Paraonidae and Spionidae) with depositivore feeding strategy. This feeding mode is associated to environments negatively affected by organic pollution (Rivero et a., 2005; Hossain 2018) and similar results were observed in Inner Magdalena station. Isla Tortuga presented high densities of depositivore polychaetes (Cossuridae and Ampharetidae). In contrast, Rio Marta station exhibited higher diversity values in turn associated with higher trophic groups such as omnivores and carnivores (Fig. 7). In general, the presence of carnivores has been associated with sandy sediments and a less impacted benthic community (Gray and Elliot, 2009).

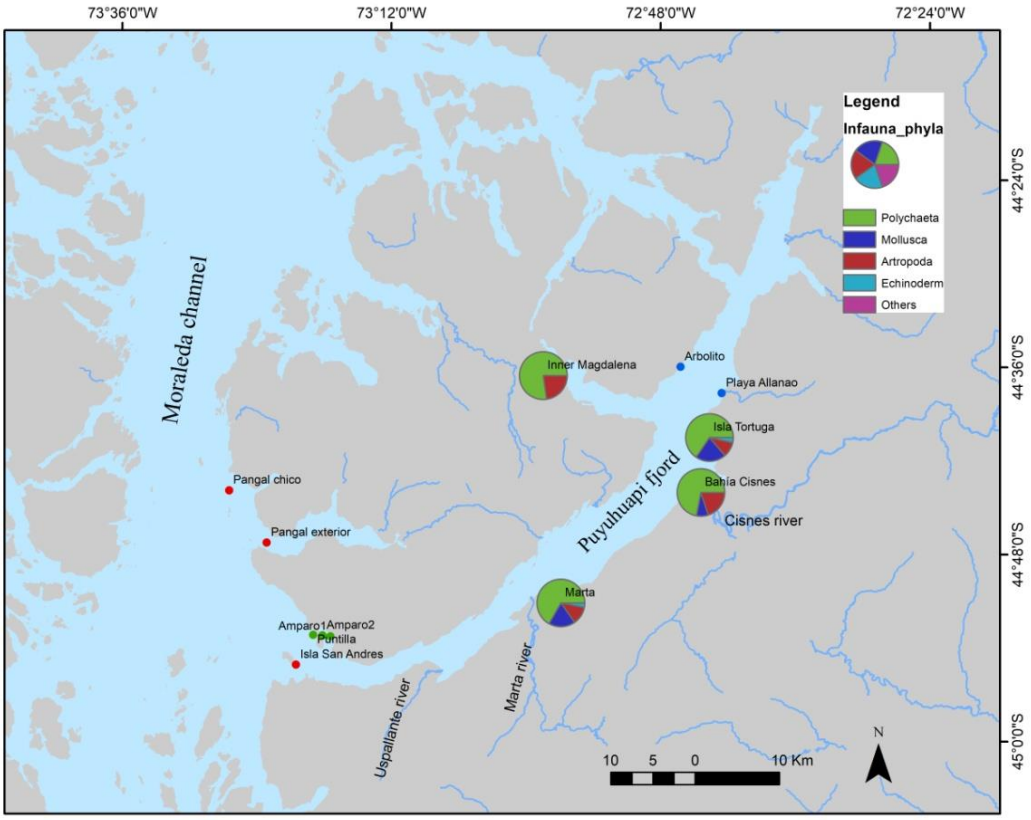

Figure 6. Spatial representation of macroinfauna phyla for stations located in middle section of the *Puyuhuapi* fjord. Green=Polychaeta, blue=Mollusca, red=Artropoda, light blue=Echinodermata and pink=Others (Nemertea, Sipunculida, Priapulida).

Table 3. List of macroinfauna recorded in soft bottom stations of the *Puyuhuapi* fjord; indicating trophic group (feeding mode), mean abundance and standard deviation (SD) for each taxa/species. Feeding modes; SDF, surface deposit feeder; SSDF, subsurface deposit feeder; CA, carnivore; OM, omnivore; SS, suspension-filter feeder.

Stations		Isla Tortuga		Inner Seno Magdalena		Bahía Cisnes		Río Marta	
Taxa/Species	Trophic group	Mean Abundance (ind/m^2)	SD	Mean Abundance (ind/m^2)	SD	Mean Abundance (ind/m^2)	SD	Mean Abundance (ind/m^2)	SD
Polychaeta									
Cossuridae	SSDF	3380	1388	25	38	50	62	0	0
Ampharetidae	SDF	2500	1101	15	30	540	183	45	66
Sabellidae	SS	1093	550	0	0	225	44	10	20
Lumbrineridae	CA	567	61	60	37	125	85	50	42
Nephtyidae	CA	20	20	135	82	205	68	75	70
Cirratulidae	SDF	353	405	1850	231	940	321	535	553
Orbiniidae	SSDF	647	281	0	0	35	30	0	0
Paraonidae	SDF	600	386	855	385	3640	309	40	37
Spionidae	SDF	267	255	475	82	2675	2814	85	94
Capitellidae	SSDF	420	106	1510	432	3720	1051	40	80
Polynoidae	CA	73	12	15	19	70	20	15	19
Gonianidae	CA	73	50	15	10	265	50	75	57
Scalibregmatidae	SSDF	73	50	0	0	0	0	25	50
Terebellidae	SDF	100	72	20	28	60	37	35	47
Nereididae	OM	13	12	15	19	95	41	5	10
Maldanidae	SSDF	47	50	50	20	25	10	80	135
Hesionidae	OM	93	31	35	30	50	53	5	10
Dorvilleidae	OM-SSDF	7	12	10	12	15	30	5	10
Phyllodocidae	CA	7	12	10	12	0	0	5	10
Sphaerodoridae	SDF	0	0	5	10	30	26	0	0
Syllidae	OM	0	0	0	0	45	41	875	886
Pectinaridae	SSDF	0	0	0	0	5	10	20	16
Magelonidae	SDF	0	0	0	0	0	0	85	93
Ophellidae	SSDF	0	0	0	0	0	0	5	10
Sigalionidae	CA	0	0	0	0	0	0	5	10
Mollusca									
Macoma sp	SS	107	50	0	0	420	494	15	19
Thyasira patagonica	SS	7	12	0	0	40	67	40	37
Nassarius sp	OM	33	23	0	0	0	0	0	0
Caecum chilensis	SDF	93	42	0	0	0	0	0	0
Cyclocardia	SS	0	0	0	0	0	0	10	12
Tindariidae	SS	0	0	0	0	0	0	5	10
Mytilidae	SS	53	12	0	0	20	28	10	12
Arthropoda									
Amphipoda	OM	53	23	80	43	35	10	40	57
Cumacea	SDF	7	12	35	19	60	28	115	156
Ostracoda	SS	13	12	685	150	1170	659	80	75
Copepoda	OM	0	0	15	19	20	28	0	0
Isopoda	OM	0	0	5	10	0	0	25	50
Tanaidacea	SDF	0	0	0	0			0	0
Others									
Ophiuroidea	SDF	20	20	0	0	0	0	0	0
Tripylaster philippii	SSDF	0	0	0	0	0	0	5	10
Priapulida	SDF	27	23	45	50	160	188	20	16
Sipunculida	SSDF	0	0	10	12	40	57	10	20
Nemertea	CA	73	12	50	60	200	92	545	796
Mean Total Abundance ± SD		10820	4518	6025	1132	15055	4782	3040	2048

Our environmental data showed marked differences in the content of total organic matter and potential redox in surface sediments of Isla Tortuga and Rio Marta (Table 4). High values of total organic matter and low values of redox potential were associated with a community dominated by small-bodied polychaetes, indicating some degree of organic pollution in Isla Tortuga station. In contrast, low content of total organic matter and high values of ORP in Rio Marta were associated with unpolluted sites.

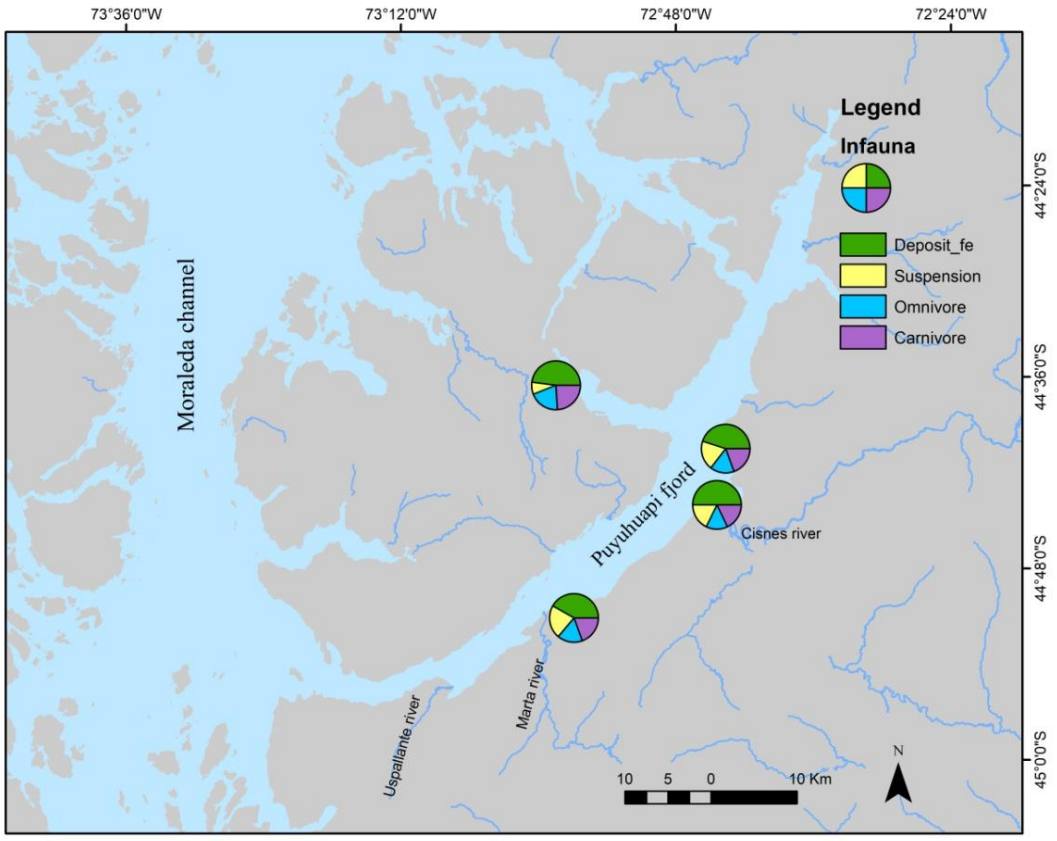

Figure 7. Spatial representation of macroinfauna trophic groups for stations located in middle section of the *Puyuhuapi* fjord. Green=deposit feeders, yellow= filter-suspension feeders, light blue=omnivores and purple=carnivores.

Table 4. Sedimentary parameters for Isla Tortuga, Arbolito and Rio Marta stations located in the middle section of the *Puyuhuapi* fjord.

Variables/Station	Isla Tortuga	Arbolito	Río Marta
Date	17.01.2020	18.01.2020	20.01.2020
Depth (m)	28	17	34
Substrate type	Soft (mud and sand)	Mixed (sand and rock)	Mixed (sand and rock)
Total organic matter (%)	4.57 ± 1.45	2.20 ± 0.16	2.33 ± 0.03
pH	6.86 ± 0.26	6.81 ± 0.11	6.77 ± 0.12
Oxide-reduction potential (mV)	45 ± 26	143 ± 22	334 ± 75

Discussion and Conclusions

Epibenthic communities

Our results agree with described Betti *et al.* (2017, 2021), describing epibenthic communities with high values of richness of species, dominated by heterotrophic suspension feeders, and well-defined aggregate communities with high biomass and diversity in well-flushed sites and on steep hard substrata where sediment stress is low. In this sense, highlight the records of stony corals (*D. dianthus*) that showed aggregations associated with greater depths (40-70 m) and in sections of greater oceanic influence in the *Puyuhuapi* fjord. In this fjord is common to found habitat-forming organisms and extensive hard bottom megabenthic aggregations (e.g. aggregations of polychaetes, porifera and sea urchins, gorgonian forests, *Aulacomya atra* belts, *Metridium* sp. and holothurian gardens; Betti et al., 2021). These communities also called marine animal forests (Forsterra et al., 2017; Rossi et al., 2017) increase the available substrate surface offering a new habitat for many kinds of sessile and mobile benthic organisms and developing a trophic strategy mostly filter-feeding (Häussermann & Försterra, 2009, 2015; Försterra et al., 2016; Betti et al., 2017, 202; Cardenas and Montiel, 2017). The suspension feeders were frequently observed in the stations located in the mouth and outer compared to middle section of the *Puyuhuapi* fjord, and were one of most diverse group, included many sponges, octocorals, bivalves, polychaetes, bryozoans, holothuroids, and ascidians. In fact, the suspension or filter-feeders are key and dominant component of benthic communities (Häussermann & Försterra, 2009; Betti et al., 2017, 2021), because

they mobilize an important fraction of the fresh OM derived from primary production and play a fundamental role transferring matter and energy from the pelagic to the benthic ecosystems. In our study area, we found in selected stations higher concentrations of suspension materials, particularly in the Rio Marta, in turn associated to high abundance of sessile organisms mainly encrusting porifera and octocorales (suspension feeders), as well as mollusks, polychaetes and echinoderms, registering a great diversity of feeding guilds (suspension feeders, depositivores, omnivores and carnivores). It is known that in some Patagonian fjords the suspended material may influence the distribution of benthic epifauna, favoring greater diversity in areas with low sedimentation (Betti et al., 2017, 2021). We suggest that in these environments, the complexity of substrates (mud, sand, detritus, rock) and allochthonous food sources (i.e. terrestrial organic matter from Marta river), which may favor feeding modes such as depositivores and some suspension feeders.

Soft bottom communities

Changes in benthic macroinfauna structure (e.g. succession or colonization events) by environmental stress such as organic enrichment, have been widely described in coastal ecosystems (Pearson and Rosenberg, 1978; Clarke & Ainsworth, 1993; Rosenberg, 2001). In Patagonian fjords, the main environment conditions forcing are closely related to the temporal and spatial variability of freshwater dynamic from rivers and glaciers (Quiroga et al., 2016; Zapata-Hernández et al., 2016; Cari et al., 2020). This freshwater input generates large supplies of terrestrial organic matter (Cari et al., 2020; Quiroga et al., 2016; Włodarska-Kowalczuk et al., 2012; Davies et al., 2020). Our results showed a spatial response of the macroinfauna, probably associated with the inputs of allochthonous organic matter (e.g. tOM, urban discharges, salmon farming waste). In fact, in Bahia Cisnes and Isla Tortuga stations exhibited functional traits (i.e. high abundances of families of opportunistic polychaetes and pollution-tolerant taxa) associated with disturbed environments. Moreover, these stations are close to the river mouth (Cisnes River), and to one of the most important urban centers in *Puyuhuapi* fjord (Puerto Cisnes, ~ 5000 inhabitants) with aquaculture activities for many years. It is important to mention that a clear example of extreme effects of organic pollution was observed in the sediments covered by an anaerobic microbiota, forming biofilms or microbial mats, in mouth of Anita River in Playa Allanao station located in the middle section of the *Puyuhuapi* fjord.

The organic pollution in sedimentary environments appear to be associated with accumulation of dissolved and particulate organic matter, nutrients in the water column and sediments, generating organic enrichment of sediments, and reduced availability of dissolved oxygen (Soto and Norambuena, 2004; Buschmann et al., 2009; Niklitschek et al., 2013; Sanchis et al., 2021). In the *Puyuhuapi* fjord, research on the effect of salmon farming on benthic systems is scarce and there are no environmental assessments according to the established by the environmental authority for the monitoring of the aquaculture industry (RAMA, 2001).

Given the natural conditions in the *Puyuhuapi* fjord, such as the different sources of OM (autochthonous and allochthonous) (Silva et al., 2011; Sepulveda et al., 2011; Montero 2017), anoxic conditions reported in the water column (Silva et al. 2014; Perez Santos 2018) and low ventilation of the deep waters product of micro-basins (Schneider et al., 2014), the incorporation of allochthonous OM to the ecosystem is a key topic in order to protect their structure and functioning.

Our results show a benthic community with high values of richness species, including several habitat types (hard and soft-bottoms). In this fjord, also we found, multiple environmental variables which define the composition and distribution of benthic communities; being the depth, the substrate inclination and lateral coupling (land-ocean influence) determining for the epifauna, and the contributions of organic sources (e.g. tOM). In this ecosystem, suspension feeders are dominating in hard bottoms and depositivores in soft bottoms. These functional traits respond to important ecosystem functions performed by these communities, such as the role of benthic organisms in OM cycles, which can have important effects on the carbon and nutrients cycle in fjord ecosystems.

Finally, the different anthropogenic activities (e.g. salmon farming and benthic fisheries) added to the natural conditions of the fjord (e.g. glaciofluvial discharges with high contributions of tOM and anoxic conditions in water column), act as forcing of changes in the structure and composition of the benthic communities. The spatial and functional information of these communities are a fundamental input for a marine spatial planning approach (Ehler and Douvere, 2009; Kyprioti et al., 2021; O´Learly et al., 2021), which must urgently be incorporated into the planning of the coastal zone in the Chilean Patagonia.

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Supplementary Data_Chapter 1

This section contains additional data of the classification benthic environments evaluated with ROV images in the Puyuhuapi fjord. The information for each station correspond to; a summary table indicating the name of the station, sector, section of fjord, date, geographical coordinates and depth range. Another table indicating the description of the type of substrate, substrate inclination (horizontal, sloping or vertical) and taxa/species registered in each transect, which were visually evaluated. Additionally, the relative presence is indicated for each taxa/specie, which was divided into high (+++: observed repeatedly), medium (++: observed more than 2 times) or low presence (+: observed only once). Finally, a set of photographs of the representative epibenthic fauna for each station is shown.

The stations are listed in the following order:

1. Pangal Exterior station (Tables 1-2 and Figure 1)
2. Isla San Andres station (Tables 3-4 and Figure 2)
3. Pangal Chico station (Tables 5-6 and Figure 3)
4. Isla Tortuga station (Tables 7-8 and Figure 4)
5. Playa Allanao station (Tables 9-10 and Figure 5)
6. Arbolito station (Tables 11-12 and Figure 6)
7. Rio Marta station (Tables 13-14 and Figure 7)
8. Puntilla station (Tables 15-16 and Figures 8-9)
9. Amparo-1 (Tables 17-18 and Figure 10)
10. Amparo-2 (Table 19-20 and Figure 11)
11. Inner Seno Magdalena (Table 21-22 and Figure 12)

1. PANGAL EXTERIOR STATION

Table 1. Station, date, geographic position, depth range of the record with ROV in Pangal exterior, Magdalena Island National Park (PNIM), March 2019.

Site	**ROV Pangal exterior**
Sector/Section fjord	**PNIM/Outer**
Code	Station 1
Date	07-03-2019
Latitude (°S)	44°48'29.99"
Longitude (°W)	73°22'13.08"
Depth Range (m)	64-92

Table 2. Type of substrate and list of taxa/species identified in station Pangal exterior, Magdalena Island National Park (PNIM), March 2019.

Depth (m)	**64-74**	**74-84**	**84-92**
Substrate	Mud + Organic detritus + Shells cover + rocks	Mud + Organic detritus + Shells cover + rocks	Mud + Organic detritus + Shells cover + rocks
Substrate inclination	Horizontal	Horizontal/ Sloping	Horizontal
Taxa/species	+ Porifera 15 + *Bolocera* sp + *Actinostola chilensis* ++ *Mesozoanthus fossii* + *Primnoella chilensis* +++ *Desmophyllum dianthus* +++ Bivalvia 1 ++ Serpulidae + *Chaetopterus variopedatus* + Terebellidae 1 + *Aspidostoma giganteum* + *Psolus* sp +++ *Loxechinus albus* + Asteroidea 5 + *Prolatilus jugularis* ++ *Patagonotothen* sp	+ *Thouarella* sp + *Primnoella chilensis* + Serpulidae + *Psolus* sp +++ *Loxechinus albus*	+ Porifera 17 + *Mesozoanthus fossii* + Asteroidea 5 + *Psolus* sp +++ *Loxechinus albus* + *Prolatilus jugularis*

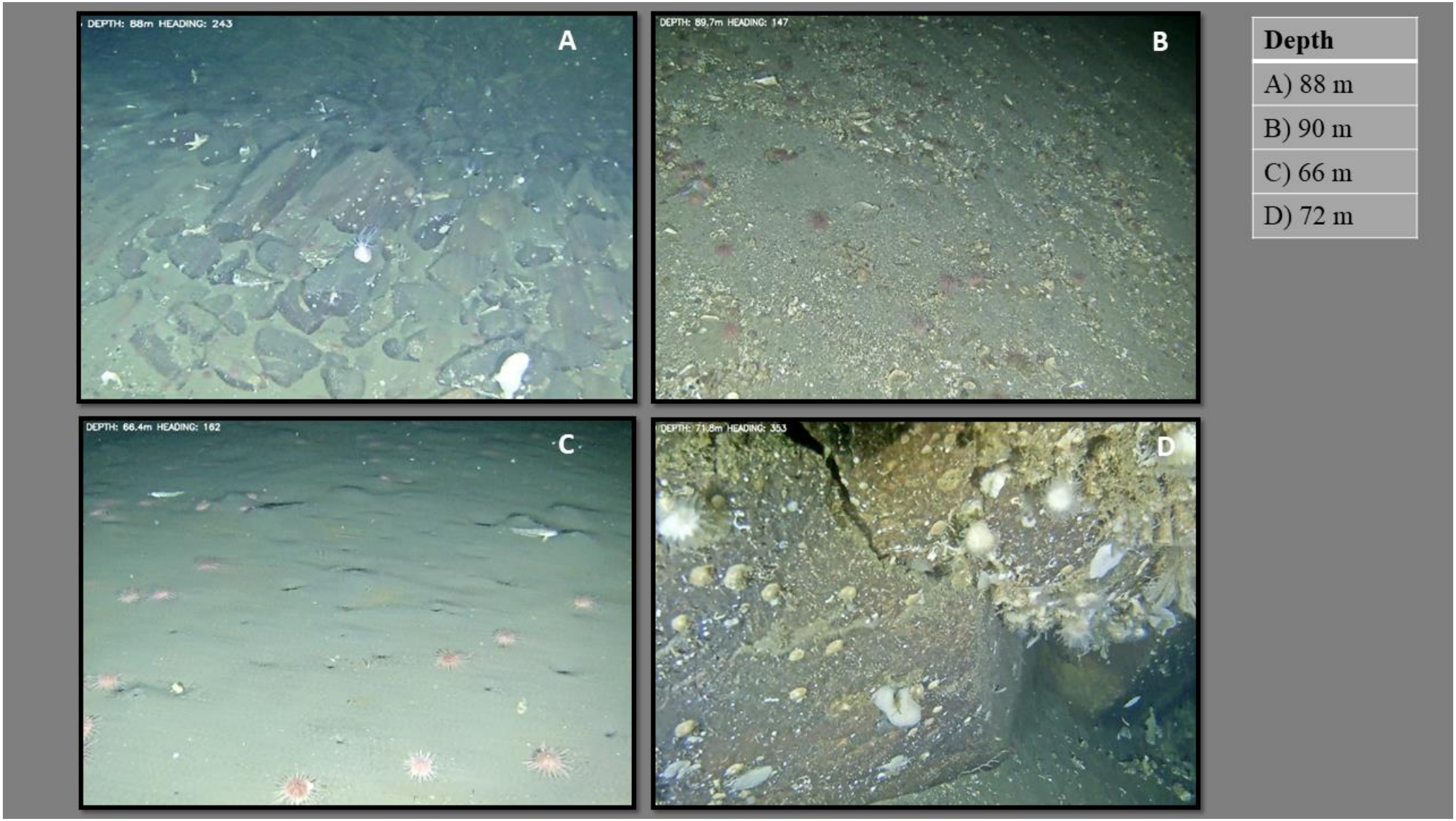

Figure 1. ROV images, Pangal Exterior. A) Rock bottom with mud, *Psolus* sp and Asteroidea sp. B) Sandy bottom with debris of shells, *Loxechinus albus* is observed. C) Mud bottom; *Loxechinus albus* and the fish *Prolatilus jugularis* are observed. D) Stony corals and encrusting sponges.

2. ISLA SAN ANDRES STATION

Table 3. Station, date, geographic position, depth range of the record with ROV in Isla San Andrés, Puerto Gaviota, March 2019.

Site	**ROV Isla San Andrés**
Sector/Section fjord	Puerto Gaviota/Outer
Code	Station 2
Date	07-03-2019
Latitude (°S)	44°56'17.556"
Longitude (°W)	73°19'21.215"
Depth Range (m)	0-28

Table 4. Type of substrate and list of taxa/species identified in station Isla San Andrés, Puerto Gaviota, March 2019.

Depth (m)	**0-12**	**12-20**	**20-28**
Substrate	Mud + Rocks with coralligenous algae + Shells cover	Rocks with coralligenous algae	Rocks with coralligenous algae
Substrate inclination	Sloping	Sloping	Sloping
Taxa/species	+ *Macrocystis pirifera* ++ Porifera 6 + *Cliona chilensis* +++ *Metridium senile* +++ *Primnoella chilensis* ++ Bivalvia 1 + *Chiton bowenii* + Serpulidae + *Munida gregaria* + Asteroidea 6 + *Cosmasterias lurida* + *Glabraster antarctica* ++ *Arbacia dufresnii* +++ *Loxechinus albus* +++ *Heterocucumis godeffroyi* + Ascidiacea 1 + *Pinguipes chilensis*	+ Porifera 6 + Porifera 2 + Porifera 16 + Porifera 17 + Porifera 19 + *Axinella crinita* + *Cliona chilensis* + *Primnoella chilensis* + *Zygochlamys patagonica* + Serpulidae + Sabellidae + *Cosmasterias lurida* + *Glabraster antarctica* + Asteroidea 6 +++ *Heterocucumis godeffroyi* + *Psolus* sp + *Arbacia dufresnii* + Ascidiacea 1 + *Sycozoa* sp + *Sebastes oculatus* + *Pinguipes chilensis*	+ Porifera 16 + *Bolocera* sp + *Primnoella chilensis* ++ *Zygochlamys patagonica* + Bivalvia 1 + Serpulidae +++ Polychaeta 1 + *Paralomis granulosa* + *Munida gregaria* + Asteroidea 7 + *Cosmasterias lurida* + *Heterocucumis godeffroyi* + *Arbacia dufresnii* + Ascidiacea 1

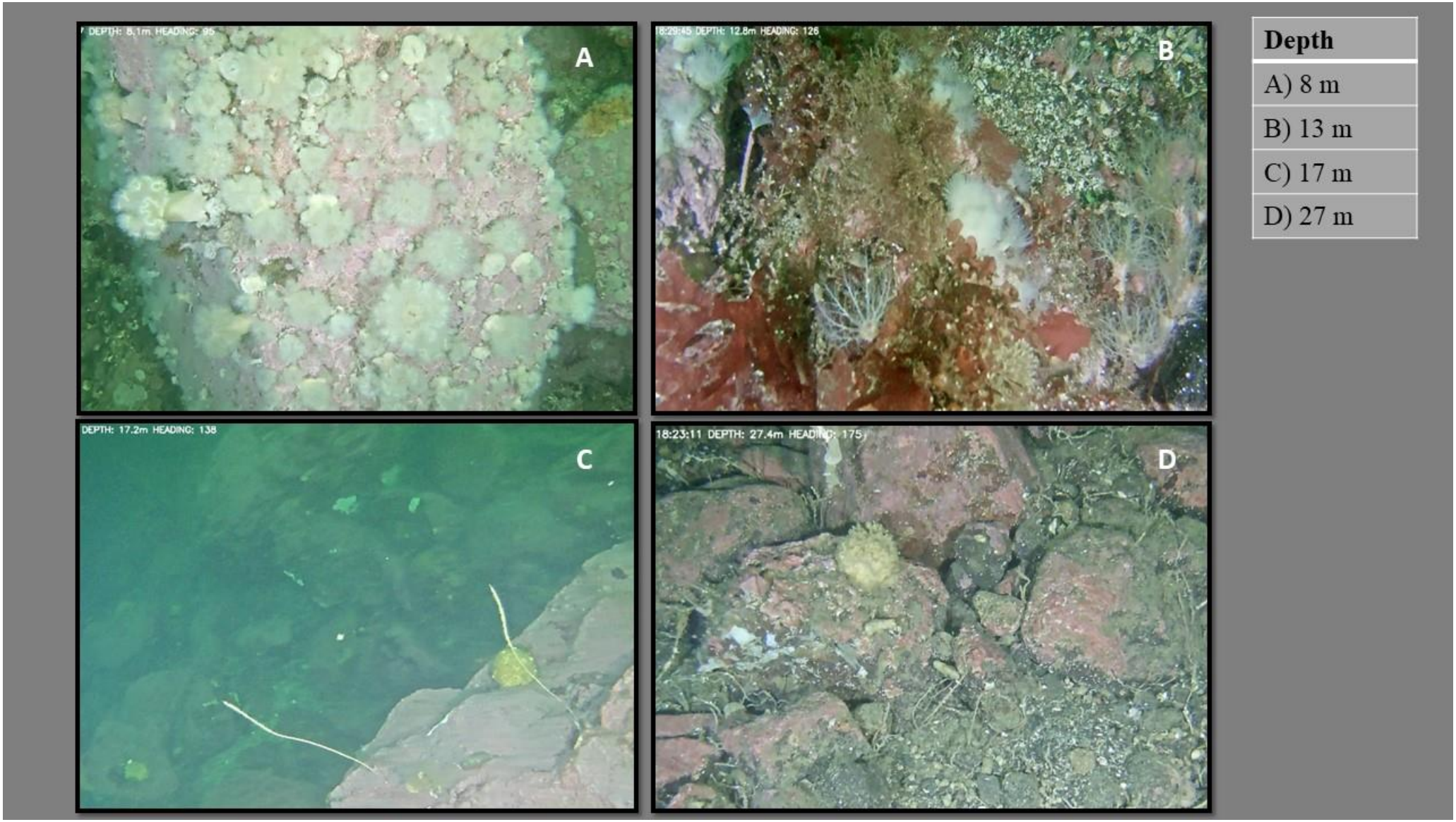

Figure 2. ROV images, Isla San Andrés. A) Rock bottom with coralligenous algae covered with *Metridium senile*. B) Rock covered mainly with *Metridium senile*, *Heterocucumis godeffroyi* and rodophyta algae. C) Rock with *Primnoella chilensis* and *Cliona chilensis*. D). Rock bottom and organic detritus, polychaete tubes, corallinales algae and unidentified sponge.

3. PANGAL CHICO STATION

Table 5. Station, date, geographic position, depth range of the record with ROV in Pangal chico, Magdalena Island National Park (PNIM), March 2019.

Site	**ROV Pangal chico**
Sector/Section fjord	PNIM/Outer
Code	Station 3
Date	08-03-2019
Latitude (°S)	44°45'11.83"
Longitude (°W)	73°25'41.34"
Depth Range (m)	36-50

Table 6. Type of substrate and list of taxa/species identified in station Pangal chico, Magdalena Island National Park (PNIM), March 2019.

Depth (m)	**36-40**	**40-44**	**44-50**
Substrate	Rocks with coralligenous algae + Organic detritus + Shells cover + Mud	Rocks with coralligenous algae + Organic detritus + Shells cover	Rocks with coralligenous algae + Organic detritus + Shells cover
Substrate inclination	Sloping	Sloping	Sloping
Taxa/species	+++ *Axinella crinita* + Porifera 15 + Porifera 18 + Porifera 19 + Porifera 20 ++ *Actinostola chilensis* + *Dactylanthus antarcticus* + Actiniaria 1 + *Desmophyllum dianthus* +++ *Primnoella chilensis* +++ *Thouarella* sp + *Alcyonium* sp + *Fusitriton magellanicus* +++ Bivalvia 1 + *Zygochlamys patagonica* + *Chaetopterus variopedatus* +++ Serpulidae ++ Polychaeta 1 +++ Terebellidae 1 +++ *Aspidostoma giganteum* + *Cellaria* sp + *Adeonella* sp + *Reteporella magellensis* + Asteroidea 6 + Asteroidea 7 + *Cosmasterias lurida* + *Arbacia dufresnii* +++ *Loxechinus albus* ++ *Heterocucumis godeffroyi* + *Psolus* sp + *Gorgonocephalus chilensis* + Ascidiacea 1 + *Patagonotothen* sp ++ *Sebastes oculatus*	++ *Axinella crinita* + Porifera 15 + *Actinostola chilensis* +++ *Thouarella* sp ++ *Primnoella chilensis* +++ *Desmophyllum dianthus* +++ Bivalvia 1 + *Zygochlamys patagonica* +++ Polichaeta 1 +++ Serpulidae +++ Terebellidae 1 + *Chaetopterus variopedatus* + *Propagurus gaudichaudi* + *Aspidostoma giganteum* + Bryozoa 4 + *Cellaria* sp + *Reteporella magellensis* + Asteroidea 7 + *Cosmasterias lurida* + *Cycethra verrucosa* + *Odontaster penicillatus* +++ *Loxechinus albus* + *Psolus* sp	+ *Axinella crinita* + *Primnoella chilensis* +++ *Thouarella* sp +++ Bivalvia 1 + *Zygochlamys patagonica* +++ *Chaetopterus variopedatus* ++ Serpulidae +++ Terebellidae 1 + *Reteporella magellensis* + *Aspidostoma giganteum* + *Glabraster antarctica* +++ *Loxechinus albus* + *Psolus* sp + *Prolatilus jugularis*

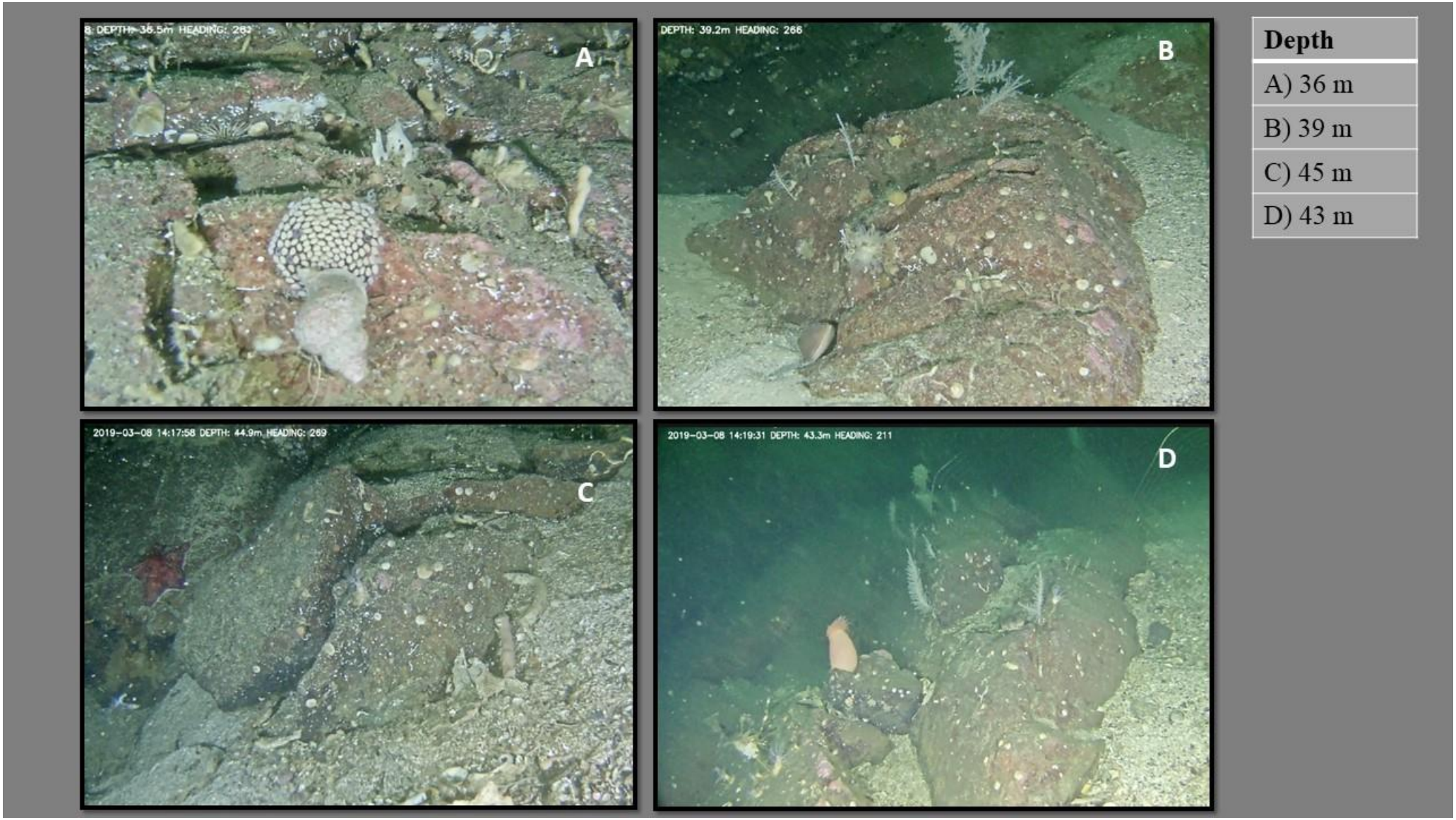

Figure 3. ROV images, Pangal Chico. A) Rocky bottom with coralligenous algae; *Fusitriton magellanicum* with oviposition, *Arbacia dufresnii* and sponge unidentified (Porifera 4). B) Rock with *Thouarella* sp., unidentified actinia and *Magellania venosa*. C) Rock with organic detritus and *Glabraster antarctica*. D) Rock with organic detritus and shells covers; *Thouarella* sp. and *Actinostola chilensis*.

4. ISLA TORTUGA STATION

Table 7. Station, date, geographic position, depth range of the record with ROV in Tortuga, Puyuhuapi fjord, January 2020.

Site	**ROV Tortuga**
Sector/Section fjord	Puerto Cisnes/Middle
Code	Station 1
Date	17-01-2020
Latitude (°S)	44°41'5.03"
Longitude (°W)	72°42'36.56"
Depth Range (m)	0.5-8.5

Table 8. Type of substrate and list of taxa/species identified in station in Tortuga, Puyuhuapi fjord, January 2020.

Depth (m)	**0.5-8.5**
Substrate	Mud + Orgacic detritus + Rocks + Shells cover
Substrate inclination	Horizontal
Taxa/Species	++ Rodophyta +++ Chlorophyta ++ *Ulva* sp +++ *Macrocystis pyrifera* + Bryozoa +++ Cirripedia ++ *Metacarcinus edwardsii* ++ *Cosmasterias lurida* +++ *Sebastes oculatus* +++ *Pinguipes chilensis*

Figure 4. ROV images, Tortuga station; A) Isla Tortuga Island, show a sandy beach. B) Bottom with rocks, green algae cover (Chlorophyta) and Cirripedia. C) Bottom with rocks, organic detritus and shells cover; Rhodophyta, *Macrocystis pyrifera*, *Cosmasterias lurida*, *Metacarcinus edwardsii* and *Pinguipes chilensis*. D) Bottom with rocks, shells cover and green algae cover (Chlorophyta and *Macrocystis pyrifera*).

5. PLAYA ALLANAO STATION

Table 9. Station, date, geographic position, depth range of the record with ROV in Playa Allanao, Puyuhuapi fjord, January 2020.

Site	**ROV Playa Allanao**
Sector/Section fjord	Puerto Cisnes/Middle
Code	Station 2
Date	17-01-2020
Latitude (°S)	44°38'12.1"
Longitude (°W)	72°41'38.7"
Depth Range (m)	6-40

Table 10. Type of substrate and list of taxa/species identified in station Playa Allanao, Puyuhuapi fjord, January 2020.

Depth (m)	**6-9**	**9-17**	**20-40**
Substrate	Sand + Mud + Organic detritus	Mud + Organic detritus + Bacterial mats	Rocks + Boulders + Mud + Organic detritus
Substrate inclination	Horizontal	Horizontal and Sloping	Horizontal
Taxa/species	++ *Metacarcinus edwardsii* ++ *Cosmasterias lurida*	++ Chlorophyta ++ Cirripedia ++ *Metacarcinus edwardsii* ++ *Cosmasterias lurida*	+++ *Metacarcinus edwarsii* ++ *Cosmasterias lurida* + *Congiopodus peruvianus* ++ Nothoteniidae

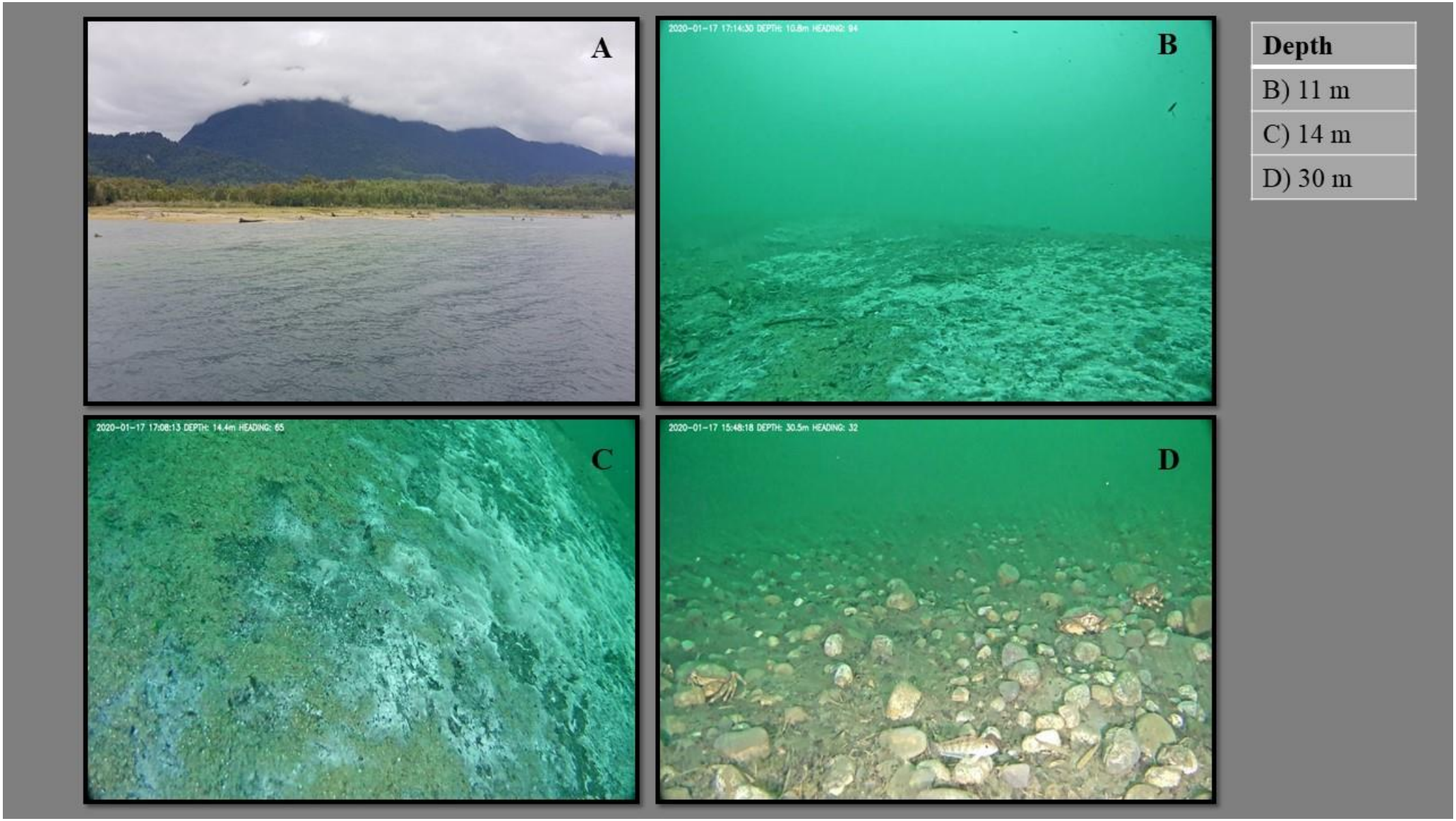

Figure 5. ROV images, Playa Allanao. A) Sandy beach and remains of terrestrial material (trees, large branches) carried by the Anita River. B-C) Soft bottom with mud, organic detritus and extensive bacterial mats D) Bottom with boulders and organic detritus, *Metacarcinus edwardsii* and Nothoteniidae fishes are observed.

6. ARBOLITO STATION

Table 11. Station, date, geographic position, depth range of the record with ROV in Sector Arbolito, Puyuhuapi fjord, January 2020.

Site	**ROV Sector Arbolito**
Sector/Section fjord	Puyuhuapi fjord/Middle
Code	Station 3
Date	18-01-2020
Latitude (°S)	44°36'35.7"
Longitude (°W)	72°45'24.56"
Depth Range (m)	5-9

Table 12. Type of substrate and list of taxa/species identified in station ROV Sector Arbolito, Puyuhuapi fjord, January 2020.

Depth (m)	**5-9**
Substrate	Mud + Organic detritus + Shells cover + Rocks
Substrate inclination	Horizontal
Taxa/species	++ Rodophyta ++ Chlorophyta + Cirripedia ++ *Metacarcinus edwardsii* +++ *Cosmaterias lurida* + Asteroidea 2 + *Sebastes oculatus*

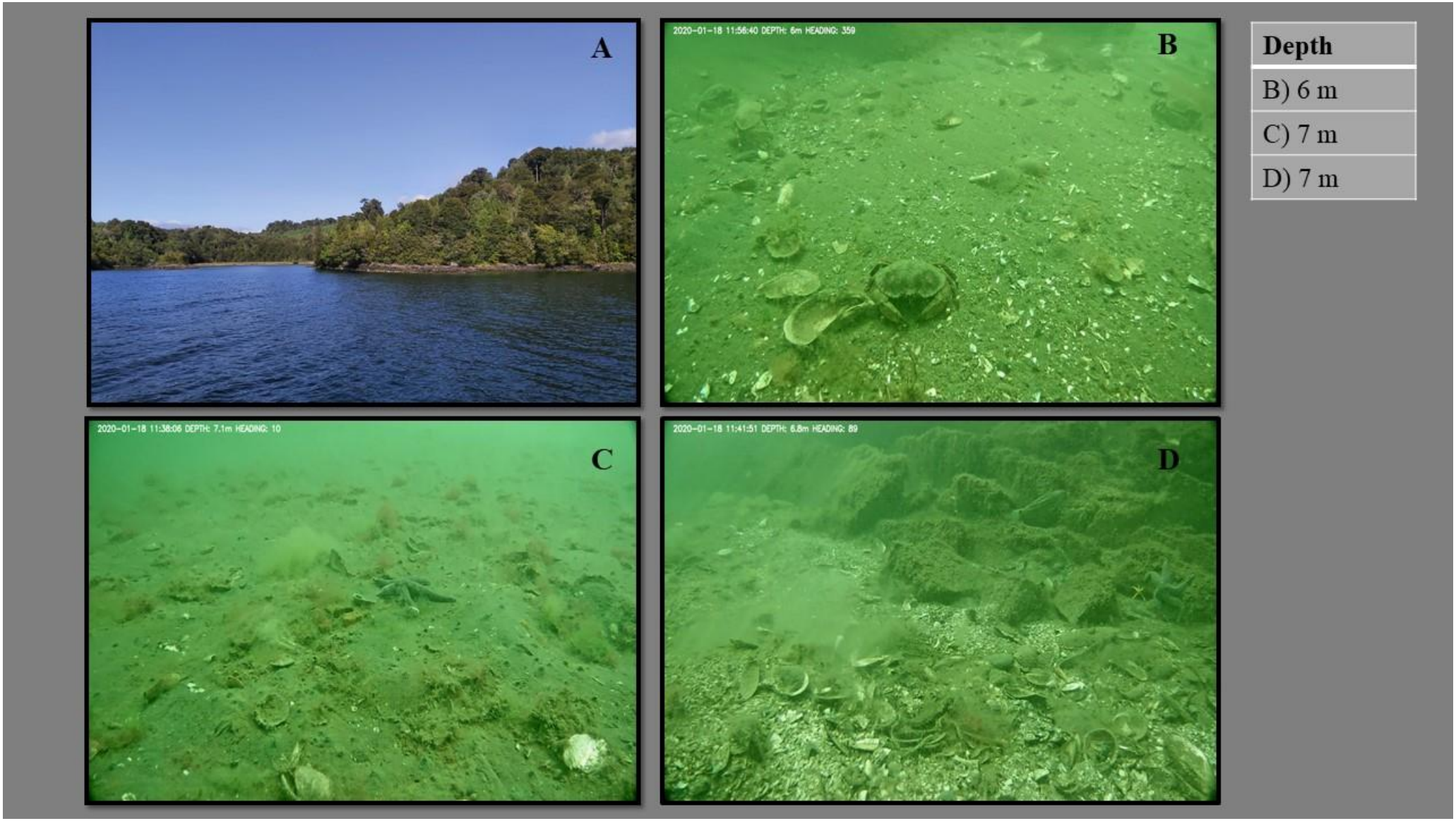

Figure 6. ROV images, Arbolito station. A) Sandy beach and dense terrestrial vegetation. B) Muddy bottom with organic detritus, shells debris and *Metacarcinus edwardsii*. B) Muddy bottom with organic detritus, shells debris and algae cover (Chlorophyta and Rodophyta); in the center a *Cosmasterias lurida*. C) Bottom with rocks, shells cover and organic detritus; *Cosmasterias lurida*, Asteroidea 3 and *Pinguipes chilensis*.

7. RIO MARTA STATION

Table 13. Station, date, geographic position, depth range of the record with ROV in River Marta, Puerto Cisnes, March 2020.

Site	**ROV River Marta**
Sector/Section fjord	Puyuapi fjord/Outer
Code	Station 7
Date	12-03-2020
Latitude (°S)	44°51'54.17"
Longitude (°W)	72°55'55.25"
Depth Range (m)	4-70

Table 14. Type of substrate and list of taxa/species identified in station ROV Río Marta, Puerto Cisnes, March 2020.

Depht (m)	**4-20**	**20-31**	**33-70**
Substrate	Rocks+ Organic detritus + Shells cover	Rocks + Rock walls + Organic detritus	Rocks+ Rock walls + Organic detritus + Shells cover + Mud
Substrate inclination	Horizontal and Sloping	Horizontal and Sloping	Sloping and Horizontal
Taxa/species	+++ Corallinales 2 + Porifera 7 +++ Porifera 1 ++ Porifera 2 ++ Porifera 3 ++ Hidrozoa + *Clavularia* sp ++ *Primnoella chilensis* ++ Cirripedia + Polychaeta 2 ++ *Aulacomya atra* ++ *Heterocucumis godefroyii* ++ *Cosmasterias lurida* +++ *Pinguipes chilensis*	+++ Corallinales 2 +++ Porifera 3 ++ Porifera 4 +++ Porifera 1 ++ Porifera 2 + Porifera 8 ++ Hidrozoa ++ *Primnoella chilensis* + *Reteporella magellensis* ++ *Cellaria* sp +++ *Aspidostoma giganteum* +++ Serpulidae + Holothuroidea ++ *Cosmasterias lurida*	+ Porifera 9 +++ Porifera 3 +++ Porifera 5 ++ Porifera 4 + Porifera10 ++ Porifera11 ++ Hidrozoa + Scleractinia + *Thoaurella* sp + *Primnoella chilensis* + Octocorallia ++ *Reteporella magellensis* ++ *Cellaria* sp ++ *Aspidostoma giganteum* ++ Serpulidae + Terebellidae 2 + Nudibranchia + Gastropoda + *Ganeria falklandica* + Asteroidea 3 + Asteroidea 4 +++ *Sebastes oculatus* + Nothoteniidae + Perciforme

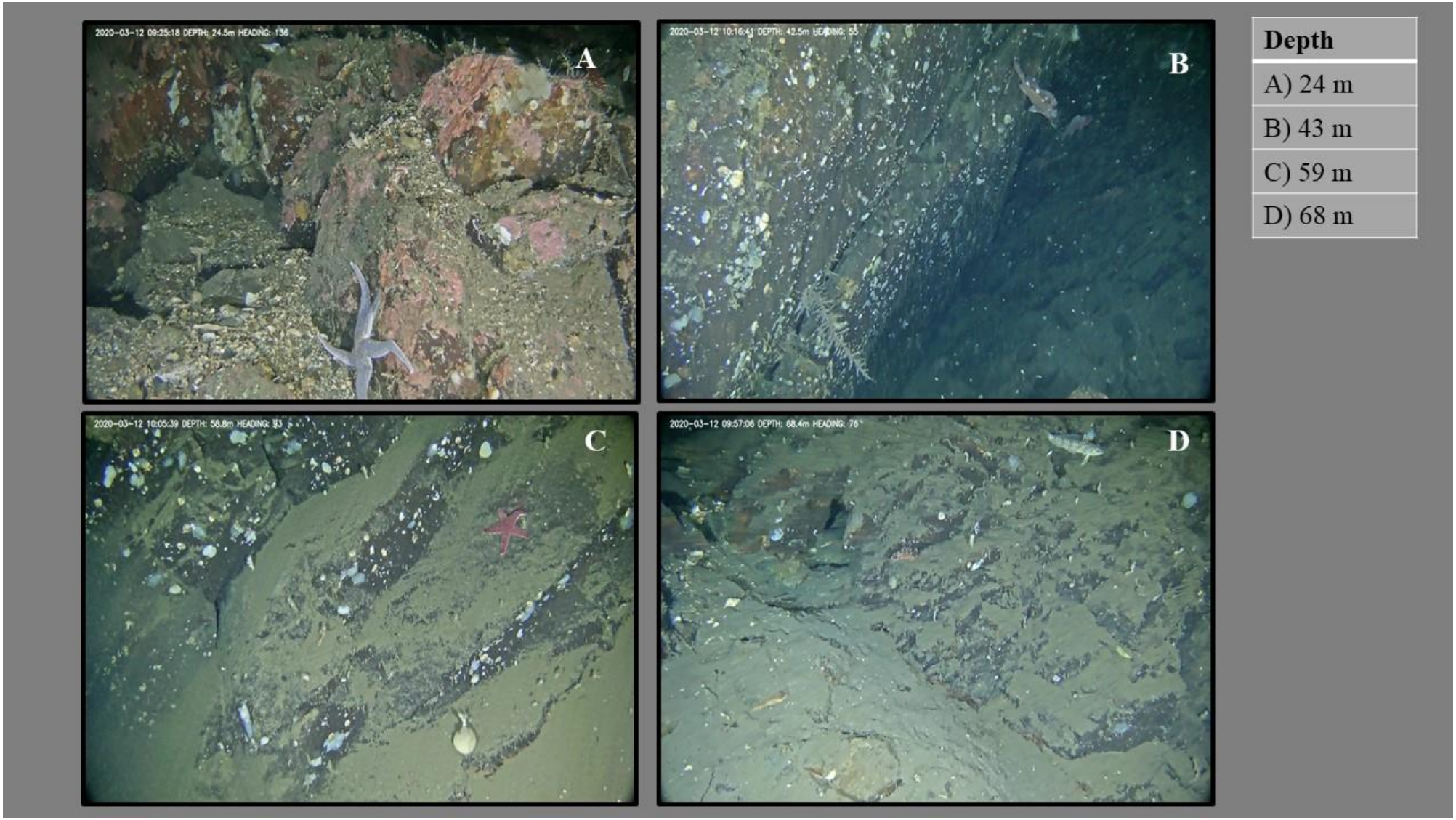

Figure 7. ROV images, Rio Marta. A) Rocks with encrusting algae (Corallinales 2), encrusting sponges (Porifera 1 and 11), tubes of Serpulidae and *Cosmasterias lurida*. B) Rocky bottom covered by organic detritus; encrusting sponges (Porifera 3, 5 and 9), *Thouarella* sp and a fish (*Sebastes oculatus)*. C) Rocky bottom covered by organic detritus and mud with encrusting sponges (Porifera 3, 5 and 9), Nudibranchia and *Ganeria falklandica*. D) Rocky bottom covered by organic detritus and mud; encrusting sponges (Porifera 3 and 5) and fishes (Nothoteniidae), *Sebastes oculatus*.

8. PUNTILLA STATION

Table 15. Station, date, geographic position, depth range of the record with ROV in Sector Puntilla, Magdalena Island National Park (PNIM), March 2020.

Site	**ROV SECTOR PUNTILLA**
Code	Station1
Sector/Section fjord	PNIM/Mouth
Date	08-03-20
Latitude (°S)	44°54'21.7"
Longitude (°W)	73°17'52.3"
Depth Range (m)	0-35

Table 16. Type of substrate and list of taxa/species identified in station ROV Sector Puntilla, Magdalena Island National Park (PNIM), March 2020.

Depth (m)	**0-10**	**10-20**	**20-30**	**30-35**
Substrate	Rock walls	Rock walls + Shells cover + Sand	Rock walls + Sand	Rocks + Sand
Substrate inclination	Vertical	Vertical/horizontal	Sloping/ horizontal	Sloping / horizontal
Taxa/species	+ Porifera 1 + Porifera 2 + Porifera 6 + Porifera 4 + *Cliona chilensis* ++ *Axinella crinita* ++ *Haliclona* sp +++ *Metridium senile* ++ *Anthotoe chilensis* + *Primnoella chilensis* + *Chiton* sp + *Cosmasterias lurida* ++ *Arbacia dufresnii* +++ *Loxechinus albus* + *Sebastes oculatus* + *Pinguipes chilensis*	++ *Tedania* sp + *Cliona chilensis* + Porifera 4 ++ *Haliclona* sp ++ *Axinella crinita* ++ *Anthotoe chilensis* +++ *Metridium senile* + Scleractinia +++ *Primnoella chilensis* + Hydrozoa + *Argobuccinum ranelliforme* + *Chiton* sp +++ *Aulacomya atra* + Nudibranchia ++ Polychaeta 2 +++ *Cosmasterias lurida* + *Glabraster antarctica* + *Meyenaster gelatinosa* +++ *Loxechinus albus* + *Arbacia dufresnii* +++ *Heterocucumis godeffroyi* + *Pinguipes chilensis*	++ Porifera 11 + *Axinella crinita* ++ *Tethya* sp +++ *Clavularia magellanica* +++ *Primnoella chilensis* + *Alcyonium* sp + Hydrozoa +++ *Magellania venosa* + *Chiton sp* + *Glabraster antarctica* + *Ganeria falklandica* + *Meyenaster gelatinosa* + Asteroidea 1 +++ *Heterocucumis godeffroyi*	+ Corallinales 1 + Corallinales 2 ++ Porifera 4 ++ Porifera 11 ++ Porifera 14 ++ *Axinella crinita* +++ *Magellania venosa* +++ *Primnoella chilensis* + *Chiton sp* + *Ganeria falklandica* + *Glabraster antarctica* + Asteroidea 1 +++ *Loxechinus albus*

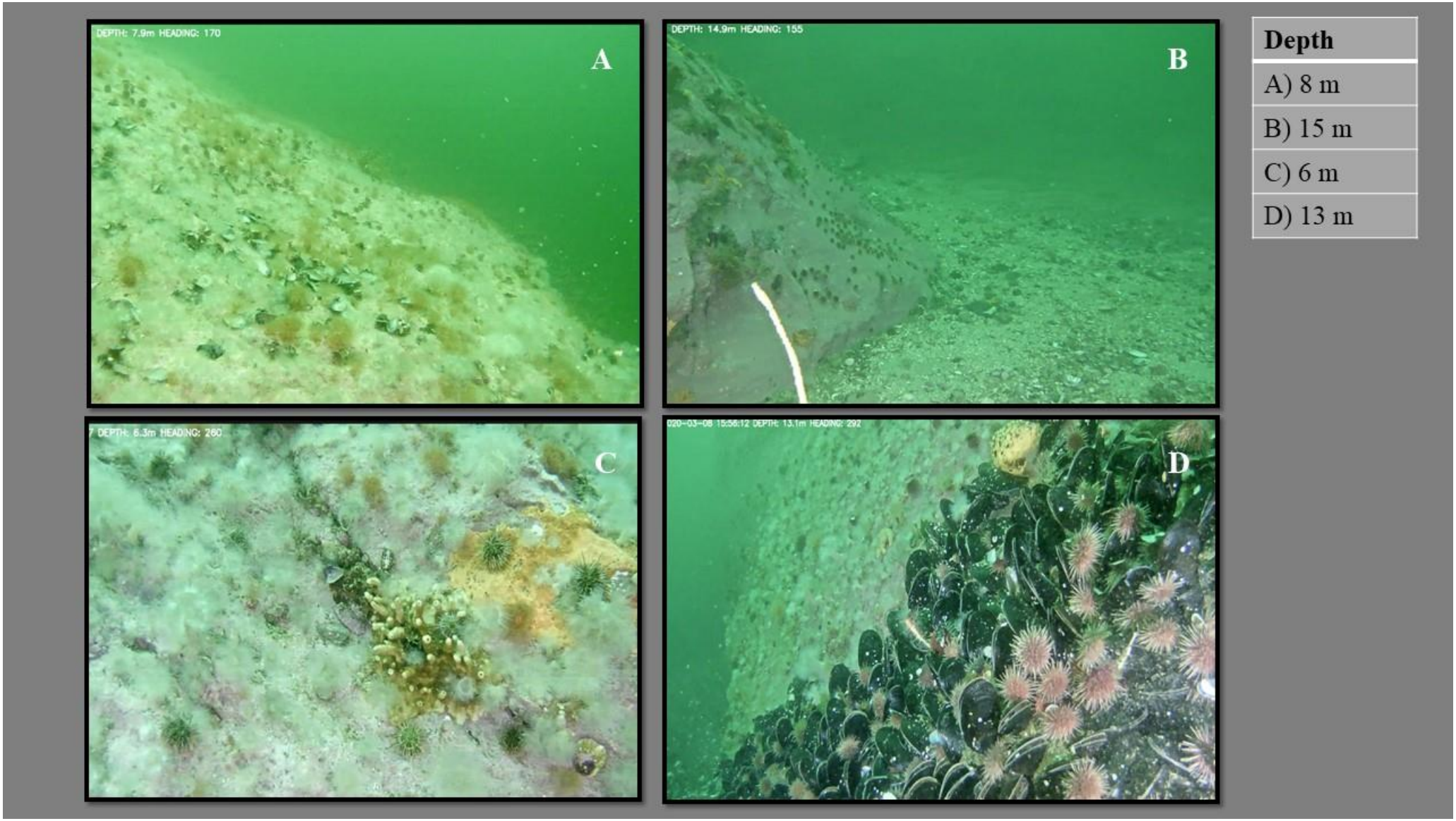

Figure 8. ROV images, Puntilla station. A) Rocky bottom covered by *Metridium seniel*. B) *Metridium senile*, *Arbacia dufresnii* and unidentified yellow sponge (Porifera 6). C) Rocks with sand and organic detritus, and *Loxechinus albus*. D) *Aulacomya atra* with *Loxechinus albus* aggregations.

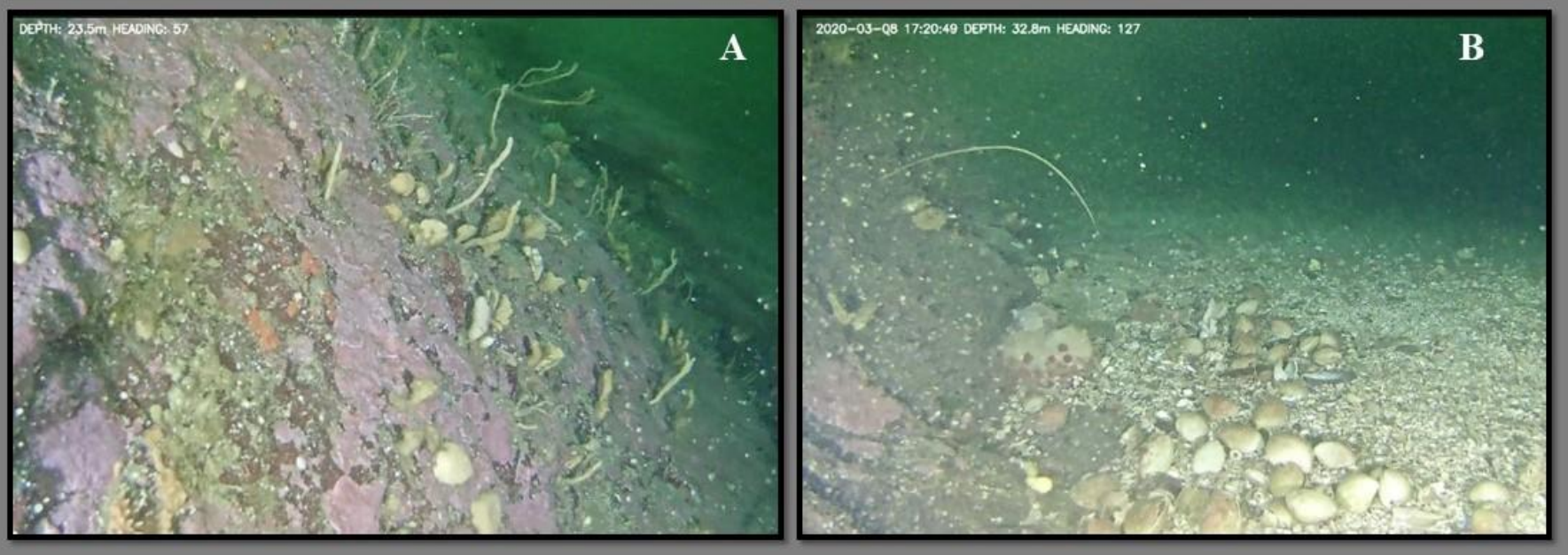

Figure 9. ROV images, Puntilla station. A) Rock bottom covered with encrusting algae (Corallinales 2) and erect sponges (Porifera 4). B) Rocky bottom with shells cover; *Loxechinus albus*, *Primnoella chilensis* and *Magellania venosa*.

9. AMPARO-1 STATION

Table 17. Station, date, geographic position, depth range of the record with ROV in Puerto Amparo-1, Magdalena Island National Park (PNIM), March 2020.

Site	**ROV AMPARO-1**
Sector/Section fjord	PNIM/Mouth
Code	Station 5
Date	10-03-2020
Latitude (°S)	44°54'26.2"
Longitude (°W)	73°16'18.35"
Depth Range (m)	0- 44

Table 18. Type of substrate and list of taxa/species identified in station ROV Puerto Amparo-1, Magdalena Island National Park (PNIM), March 2020.

Depth (m)	**0-10**	**10-20**	**20-30**	**30-44**
Substrate	Rock walls	Rocks + shells cover	Rocks	Rocks + Organic detritus + Sand
Substrate inclination	Vertical	Sloping	Sloping	Sloping/ horizontal
Taxa/species	+++ Corallinales 2 + Porifera 1 + Porifera 6 ++ Porifera 11 + *Axinella crinita* +++ *Metridium senile* + *Primnoella chilensis* + *Chiton sp* + *Tegula atra* + *Arbacia dufresnii* ++ *Cosmasterias lurida*	+++ Corallinales 2 + Porifera 1 ++ Porifera11 ++ Porifera 4 +++ *Axinella crinita* ++ *Metridium senile* ++ *Primnoella chilensis* +++ Hydrozoa + *Argobuccinum ranelliforme* ++ *Aulacomya atra* ++ *Heterocucumis godeffroyi* + Asteroidea 1 ++ *Pinguipes chilensis*	++ Corallinales 2 + Porifera 4 +++ Porifera 11 +++ *Thouarella sp* + Hydrozoa + *Aspidostoma giganteum* + Nudibranchia + *Heterocucumis godeffroyi*	++ Porifera 2 ++ Porifera 4 ++ Porifera 11 + Porifera 13 +++ *Thouarella sp* + *Primnoella chilensis* + *Clavularia sp* ++ Scleractinia + Briozoa 2 + Bryozoa 3 + *Reteporella magellanica* + *Aspidostoma giganteum* +++ *Magellania venosa* ++ Serpulidae +++ *Psolus sp* + Holothuroidea + Asteroidea 1 ++ Ascidacea 2 + Nothoteniidae

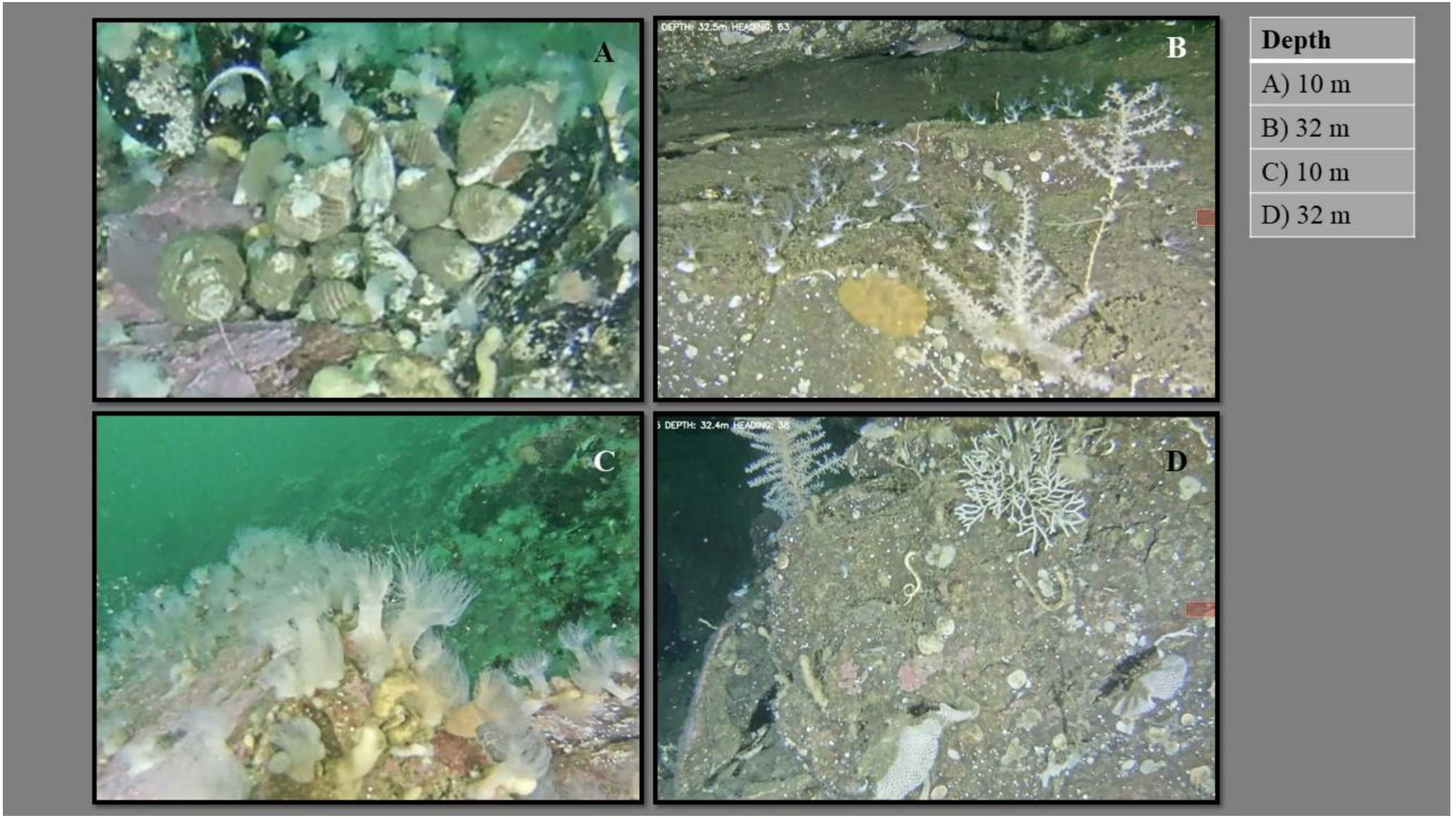

Figure 10. ROV images, Puerto Amparo-1. A) Rocky bottom, snails of the species *Argobuccinum ranelliforme*. B) Rocks with organic detritus and *Psolus* sp, *Thouarella* sp and *Sebastes oculatus.* C) Rocks with *Metridium senile*. D)Rocks with *Thouarella* sp, tubes of Serpullidae, *Reteporella magellensis* and Bryozoa 2.

10. AMPARO-2 STATION

Table 19. Station, date, geographic position, depth range of the record with ROV in Puerto Amparo-2, Magdalena Island National Park (PNIM), March 2020.

Site	**ROV AMPARO-2**
Sector/Section fjord	PNIM/Mouth
Code	Station 10
Date	11-03-2020
Latitude (°S)	44°54'51"
Longitude (°W)	73°17'0.75"
Depth Range (m)	0- 15

Table 20. Type of substrate and list of taxa/species identified in station ROV, Puerto Amparo-2, Magdalena Island National Park (PNIM), March 2020.

Depth (m)	**0-5**	**5-10**	**10-15**
Substrate	rock walls	Rocks with coralligenous algae + Sand	Rocks with coralligenous algae + Sand + Shells cover
Substrate inclination	Vertical	Sloping / horizontal	Sloping/ horizontal
Taxa/species	++ Corallinales 1 + Corallinales 2 + Porifera 2 +++ *Anthotoe chilensis* + *Nacella sp* + *Chiton sp* ++ *Crepipatella sp* +++ *Mytilus chilensis* +++ *Loxechinus albus*	+++ Corallinales 1 ++ Corallinales 2 + Porifera 2 ++ Porifera 6 ++ Porifera 11 + Porifera 4 ++ *Cliona chilensis* + *Anthotoe chilensis* ++ *Crepipatella sp* + *Chiton bowenii* + *Tegula atra* + Asteroidea 1 + *Cosmasterias lurida* +++ *Arbacia dufresnii* + *Loxecinus albus* + Holothuroidea +++ *Heterocucumis godeffroyi* + Nothoteniidae	+ Corallinales 1 +++ Corallinales 2 ++ *Axinella crinita* + *Cliona chilensis* ++ Porifera 4 ++ Porifera 11 ++ Porifera 12 + *Nacella sp* +++ Serpulidae + Asteroidea 1 + *Cosmasterias lurida* + *Glabraster antartica* +++ *Arbacia dufresnii* +++ *Heterocucumis godeffroyi*

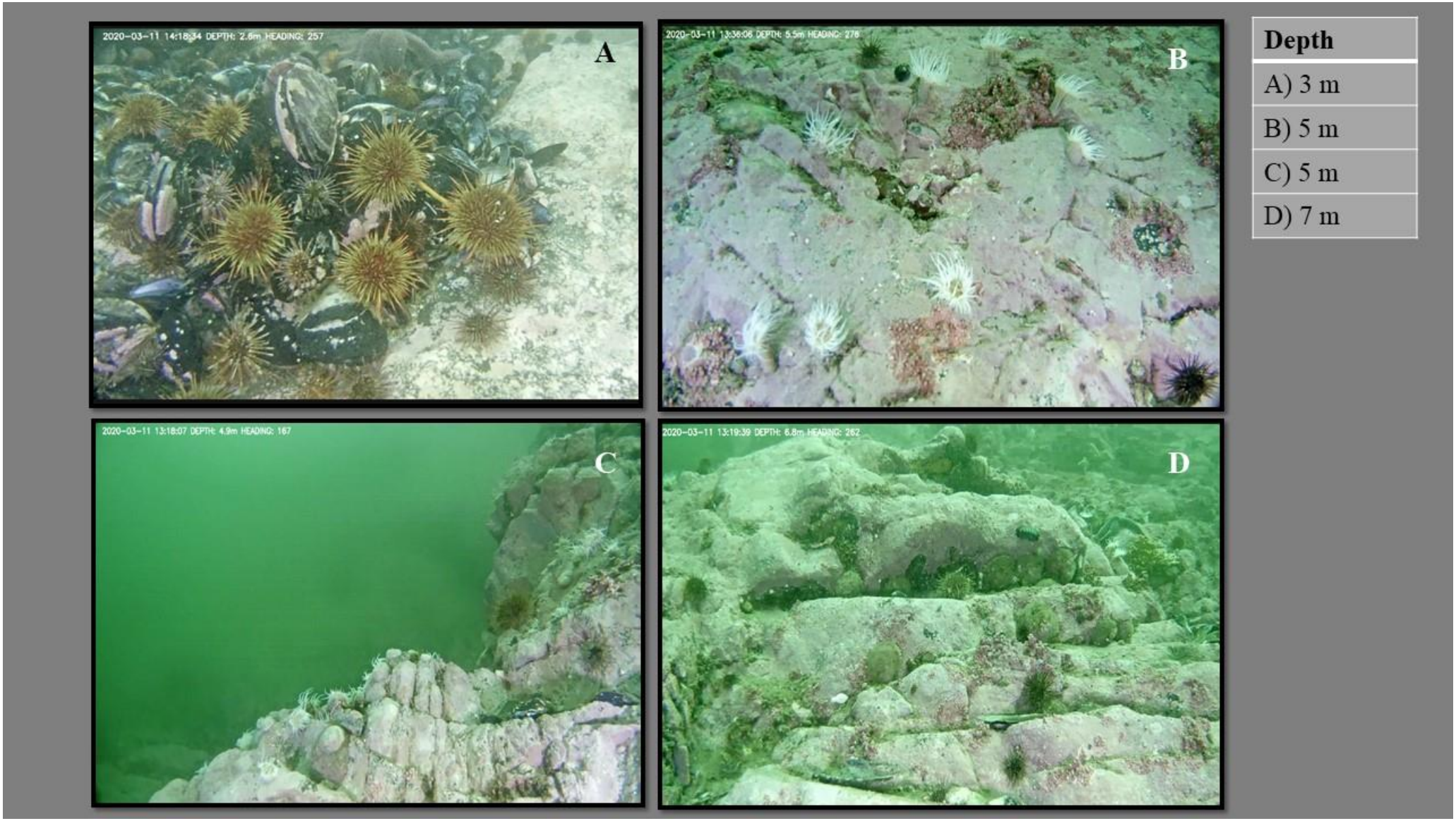

Figure 11. ROV images, Puerto Amparo-2. A) Rocky bottom; *Mytilus chilensis* and *Loxechinus albus*. B) Rocks with coralligenous algae, and *Anthothoe chilensis*, *Arbacia dufresnii* and *Loxechinus albus*. C) Rocks with coralligenous algae and *Anthothoe chilensis*. D) Rocks with coralligenous algae, Polyplacophora, *Crepipatella* sp and *Arbacia dufresnii*.

11. INNER SENO MAGDALENA STATION

Table 21. Station, date, geographic position, depth range of the record with ROV in Inner Seno Magdalena, Puyuhuapi fjord, December 2020.

Site	**ROV INNER SENO MAGDALENA**
Sector/Section fjord	Seno Magdalena/Middle
Code	Station 11
Date	12-12-2020
Latitude (°S)	44°37'22.7"
Longitude (°W)	72°57'41.91"
Depth Range (m)	25-50

Table 22. Type of substrate and list of taxa/species identified in station ROV, Inner Seno Magdalena, Puyuhuapi fjord, December 2020.

Depth (m)	**25-50**
Substrate	Boulders + Organic detritus + Mud
Substrate inclination	Horizontal
Taxa/species	+++ *Munida gregaria* + *Metacarcinus edwardsii* + Polychaeta 2 + Nothoteniidae

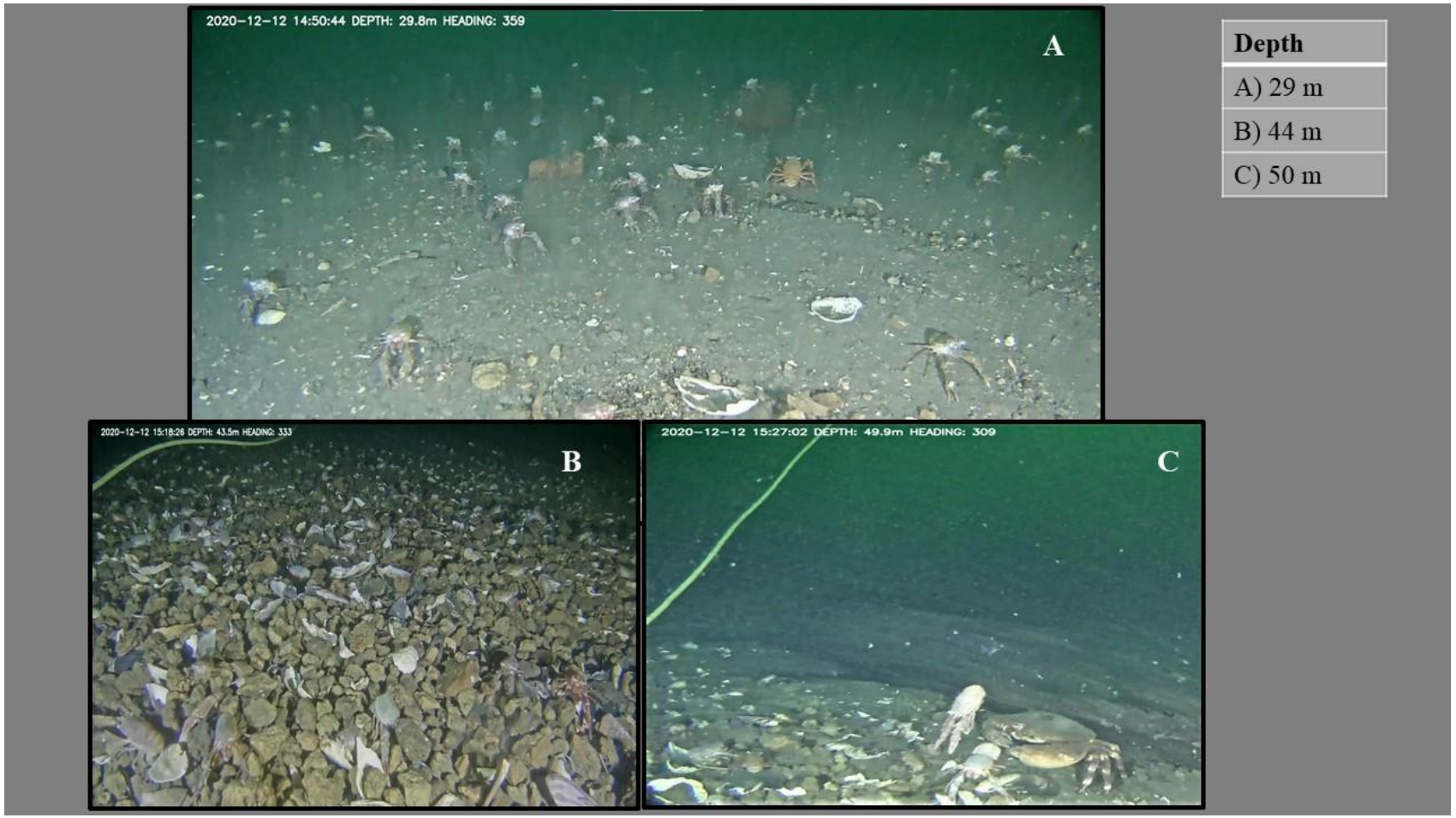

Figure 12. ROV images, Inner Seno Magdalena station. A-B) Soft bottom with mud, sand and organic detritus, and *Munida gregaria*. C) Mixed bottom with pebbles, boulders and shells, with *Munida gregaria.* D) Mixed bottom with *Munida gregaria* and *Metacarcinus edwardsii.*

Chapter 2

Trophic structure of benthic communities in a Chilean fjord (45°S) influenced by salmon aquaculture: Insights from stable isotopic signatures

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Baseline

Trophic structure of benthic communities in a Chilean fjord (45°S) influenced by salmon aquaculture: Insights from stable isotopic signatures

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ABSTRACT

Benthic marine food webs, which recycle organic matter and sustain unique biodiversity, are an important component of estuarine Patagonian fjords; however, these may be heavily influenced by salmon farming activities. Under the above conditions, this study collected several food sources (sediment organic matter, suspended particulate organic matter, macroalgae) and 26 benthic invertebrate species, and analyzed them using a Bayesian mixing model. Briefly, carbon and nitrogen stable isotopes analyses revealed that the highly variable isotopic niche widths respond to food supplies from marine and terrestrial organic matter. In addition, particulate and dissolved waste from salmon farming activities were found to modify isotopic composition ratios in many suspension feeders. Particularly, our results indicate high $\delta^{15}N$ values in the sponge *Cliona chilensis* and the encrusting coral *Incrustatus comauensis*, which appear to be potential ecological indicators for evaluating stoichiometry imbalances and trace organic pollution sources in fjord environments.

As dynamic environments, Chilean fjords (41–55°S) receive high freshwater and terrestrial organic matter (tOM) inputs from rivers and calving glaciers, resulting in a highly stratified estuarine ecosystem (Silva et al., 2011; Quiroga et al., 2016; Pérez-Santos et al., 2018; Rebolledo et al., 2019). This ecosystem is further capable of efficiently exporting organic carbon to sediments, contributing to CO_2 sink (Sepulveda et al., 2011; Torres et al., 2014; Bianchi et al., 2020). Such strong interactions between marine and terrestrial environments may, however, rapidly produce changes in nutrient and organic matter (OM) supplies (Bianchi et al., 2020). Hence, there has been a recent increase in research interest in benthic trophic structures and allochthonous food sources supply in these areas (Zapata-Hernández et al., 2014, 2016; Quiroga et al., 2016; Cari et al., 2020).

In fjord ecosystems influenced by salmon farming activities, carbon and nitrogen wastes contribute between 60 and 70% of the dissolved nutrients input (Lefebvre et al., 2001; Wang et al., 2012). Intensive salmon farming may generate organic enrichment of sediments, and reduced availability of dissolved oxygen (Soto and Norambuena, 2004; Buschmann et al., 2009; Niklitschek et al., 2013), impacting the benthic communities and diversity which are highly sensitive to organic enrichment, increasing in opportunistic and pollution-tolerant species (e.g., Keeley et al., 2019; Tičina et al., 2020; Sanchis et al., 2021). While significant, little attention has been given to allochthonous inorganic nutrient incorporation into the benthic food web, being restricted to few locations and benthic communities (Woodcock et al., 2018; Buschmann et al., 2009). Studies based on stable isotopes signatures suggest that benthic organisms of infaunal and epibenthic communities showed higher $\delta^{15}N$ values at near fish cage sites compared to sites away from the farm (Lojen et al., 2005; Wai et al., 2011; Callier et al., 2013). On the other hand, it is known that fish effluents may produce changes on functional traits in a far-field component that can be used to determine the ecological footprint left by this activity (Wang et al., 2012).

Notwithstanding, in describing fjord ecosystems, some studies have used fatty acid analysis and stable isotope analysis (e.g., Andrade et al., 2016; Riccialdelli et al., 2017; Woodcock et al., 2018; Ruiz-Ruiz et al., 2021) of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$), which is an efficient means for tracing carbon flow and trophic position in food webs (Michener and Lajtha, 2007; France, 2014) and, therefore, to isotopically distinguish among food sources (DeNiro and Epstein, 1978) or to predict the trophic level of an organism or a functional group (e.g., Michener and Lajtha, 2007; Quiroga et al., 2014).

In many Chilean fjords it is common to find habitat-forming

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organisms, e.g., *Desmophyllum dianthus* (and other hard coral facies), gorgonian forests, mussel belts, holothurian gardens and different sponges. These organisms increase available substratum surface areas, offering new habitats for many sessile and mobile benthic organisms that mostly make use of a filter-feeding trophic strategy (Häussermann and Försterra, 2009; Försterra et al., 2016; Cárdenas and Montiel, 2017; Betti et al., 2017, 2021). Because they mobilize an important portion of the fresh OM derived from primary production, these organisms dominate and are key to epibenthic communities (Häussermann and Försterra, 2009; Betti et al., 2017, 2021).

While there are scarce studies on the incorporation of allochthonous nutrients in estuarine food webs, previous data on freshwaters in southeastern Alaska described high levels of nitrogen (22–73%) and carbon (7–52%) at all trophic levels due to the presence of Pacific salmon carcasses (Chaloner et al., 2002). Notably, Mayr et al. (2014) applied carbon isotopes and C/N ratios to Patagonian fjord sediments to reconstruct carbon and nitrogen mass accumulation rates over the last twenty years, finding environmental changes associated with anthropogenic eutrophication and salmon farming waste (Quiñones et al., 2019). Clearly, however, there remains a need for further research on the incorporation and processing of nitrogen and organic carbon by benthic fauna and food web relationships. It is known that stable isotope composition is a powerful tool for tracing the fate of fish farm wastes into the benthic biota (Lojen et al., 2005; Dolenec et al., 2007; Wai et al., 2011; Callier et al., 2013; Sanz-Lázaro and Sanchez-Jerez, 2017). Although nitrogen enrichment effect studies on benthic organisms is scarce, suspension feeders such as tunicates, cnidarians and sponges have been registered δ^{15}N-enriched near the fish farms (Lojen et al., 2005; Dolenec et al., 2007; Wai et al., 2011; Callier et al., 2013). We hypothesize that benthic communities will be influenced, on far-field by fish farm wastes, in turn, cause variations in the δ^{13}C/δ^{15}N ratio stoichiometry in suspension feeders, and it will exhibit a high trophic overlap among benthic groups. As such, this study takes data from the *Puyuhuapi* fjord system – influenced by salmon farming – in order to: (i) describe the trophic structure of the subtidal benthic communities; (ii) evaluate primary food sources that support these communities using stable isotope Bayesian analyses, and (iii) identify key benthic species based on carbon and nitrogen stable isotopes as potential tracer associated to fish farm wastes.

The *Puyuhuapi* fjord, in the south of Chile (Northern Patagonia, 45°S), is ~90 km long, ~7 km wide, and average depth 250 m. At its southern section, the mouth of the fjord is connected to the *Moraleda* channel; and, in its middle, to the *Jacaf* channel (Fig. 1). The hydrography is characterized by an estuarine type of circulation with a vertical two-layer structure comprising a highly variable 5–10 m freshwater layer, and a stable and saltier sub-pycnocline layer (Schneider et al., 2014). Compared to the latter, the upper layer is highly variable, more oxygenated, less saline, and has lower nutrient concentrations of nitrate and orthophosphate (Schneider et al., 2014; Montero et al., 2017a; Pérez-Santos et al., 2018), receiving allochthonous organic matter (AOM) inputs from mainly river discharge and rainfall (up to 3000 mm y^{-1}) (DGA, 2003). The Cisnes river is one the most important freshwaters input in the study area contributing an average flow of 233 m^3s^{-1} (Prado-Fiedler and Castro, 2008). Finally, the study area is subject to intensive salmon farming: of the total 723 farm permits in the *Aysén* region (https://mapas.subpesca.cl/ideviewer/), 86 are localized in the study area, of which 57 operated during the 2018–2020 study period (36 in *Puyuhuapi* fjord and 21 in Jacaf channel).

In the study area, we obtained hydrographic data and biological samples between February 2018 and December 2020 in five campaigns considering a total of 13 stations (Fig. 1, Table 1). The stations were placed along the fjord to sketch the ecological processes taking place in the study area (i.e., inner, middle and outer fjord). This sampling scheme was proposed to distinguish the differences along the fjord influenced by freshwater input from rivers, which also may produce changes in the indices and environmental covariates variables (Quiroga et al., 2013). It is important to mention that for logistic reasons, sampling stations were not placed close to farm sites. In this study, biological samples were collected at an average distance of 4.1 ± 2.0 Km from the nearest salmon farm. This distance was calculated in each station, considering the general current direction (south-west) to determine the influence downstream of fish farm waste on the far-field. In general, the local circulation in channels and fjords was dominated by tidal currents and topography, which can be important in the distribution of dissolved and particulate organic matter (Sobarzo et al., 2018). In the study area, hydrodynamic conditions appear to be highly influenced by freshwater input, producing a typical estuarine circulation and surface water currents present direction mainly south-west, with current velocity ranging

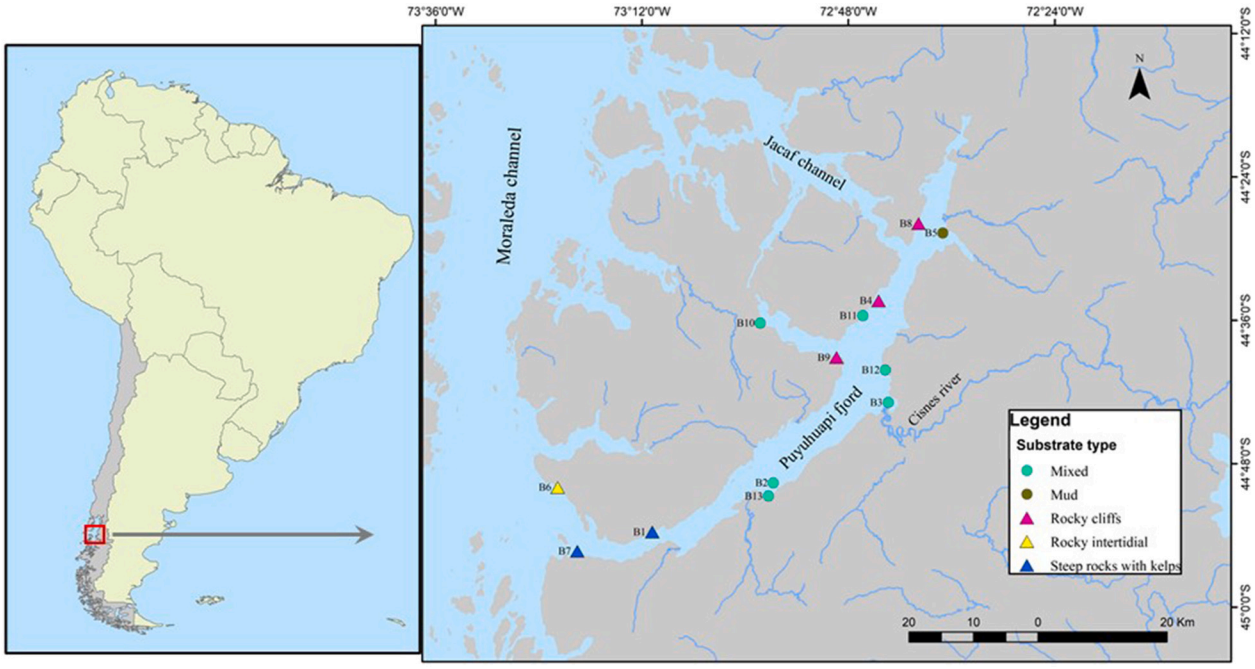

Fig. 1. Locations of the study area in *Puyuhuapi* fjord, position of the sampling stations and substrate type.

Table 1
Summary of sampling campaigns in the *Puyuhuapi* fjord, indicating the geographical coordinates, fjord section, location, sampling gear and date for each sampling station.

Station	Latitude (°S)	Longitude (°W)	Fjord section	Location	Substrate type	Depth (m)	Sampling gear	Date
B1	44°55′08.1″	73°09′28.2″	Outer	Sill	Steep rocks with kelps	35	Scuba diving	February 2018, July 2018, March 2019
B2	44°50′47″	72°55′23″	Middle	Marta river	Mixed	35	Scuba diving	February 2018, July 2018, February 2019
B3	44°43′48″	72°42′09″	Middle	Cisnes river	Mixed	30/10/20	Scuba diving/crab trap/lines fishing	July 2018, February 2019, December 2020
B4	44°35′18″	72°43′36″	Middle	Bouy	Rocky cliffs	35/20	Scuba diving/Niskin bottle	February 2018, July 2018, February 2019. For SPOM monthly time serie between March 2018 and February 2019.
B5	44°29′29.01″	72°36′19.74″	Inner	Ventisqueros river	Mud	20/20/15	Scuba diving/Van Veen grab	February 2018, July 2018, February 2019
B6	44°51′34.7″	73°20′42.7″	Outer	Gaviota Intertidial	Rocky intertidial	5	Scuba diving	February 2018
B7	44°56′52.80″	73°18′15.12″	Outer	Mouth fjord	Steep rocks with kelps	35	Scuba diving	February 2018
B8	44°28′44.88″	72°39′11.37″	Inner	Inner fjord	Rocky cliffs	35	Scuba diving	February 2018, July 2018
B9	44°40′8.81″	72°48′21.38″	Middle	Outer Seno Magdalena	Rocky cliffs	30/20	Scuba diving/lines fishing	January 2020, December 2020
B10	44°37′26.46″	72°57′22.79″	Middle	Inner Seno Magdalena	Mixed	21/20	Van Veen grab/crab trap	January 2020, December 2020
B11	44°36′35.7″	72°45′24.56″	Middle	Sector Arbolito	Mixed	17	Van Veen grab	January 2020
B12	44°41′5.03″	72°42′36.56″	Middle	Punta Tortuga	Mixed	20/28/17	Scuba diving/Van Veen grab	January 2020
B13	44°51′54.17″	72°55′55.25″	Middle	Marta river	Mixed	30/20/34	Scuba diving/lines fishing/Van Veen grab	January 2020

from 0.015 and 0.228 m s^{-1} in the first 50 m of depth, the highest velocity (~ 0.2 m s^{-1}) has been recorded on surface waters (0–10 m) in the outer section of the fjord (http://chonos.ifop.cl/atlas3/view/aysen).

Biological samples were taken between 0 and 35 m depth, from mainly mixed bottom (sand and rock) and hard bottom (rocky cliffs and steep rocks) substrates. Suspended particulate organic matter (SPOM, e.g., microorganisms, particulate organic matter and detritus), were collected monthly between March 2018 and February 2019, in discrete-depths (2, 10 and 20 m), to describe the marine and terrestrial OM temporal variability in the upper-layer in the middle fjord (B4 station). The 20 m depth layer approximately corresponded to the 1% light depth measured during the productive season (Montero et al., 2017b). SPOM were collected in Niskin bottles (10*L*), and at each depth 1500–2000 ml of seawater were filtered through pre-combusted (450 °C, 4 h) GF/F glass fiber filter (0.7 mm pore size, 47 mm diameter). Two replicates were obtained for each depth, and filters were kept frozen (−20 °C) prior to analysis of carbon and nitrogen isotope ratios. Macroalgae and benthic organisms were collected by SCUBA diving (depth < 30 m). Macroalgae were divided into three groups: (1) green algae, represented by *Ulva* spp. and *Rhizoclonium* spp.; (2) brown algae, represented by *Macrocystis pyrifera*; and (3) red algae, represented by *Agarophyton chilense* and unidentified Gigartinaceae, and collected in the maximum primary productivity period (i.e., summer). Sediment organic matter (SOM) and benthic organisms were sampled seasonally (i.e., summer and winter). SOM (0–5 cm layer) were collected from independent replicates (3–6 samples) using a modified Van Veen grab (0.05 m^2), labelled, and kept frozen (−20 °C) prior to carbon and nitrogen isotope analysis for sediment organic matter. Biological material was washed with distilled water and frozen (−20 °C) for subsequent treatment and laboratory analysis. Benthic invertebrates were kept alive with filtered seawater (0.2 μm), left to sit for 24 h to evacuate gut contents, then rinsed with distilled water and frozen (−20 °C) until further analysis. Fishes were collected using fishing lines (depth < 20 m) with dead fish (*Sebastes oculatus*) as bait. Crustaceans (*Metacarcinus edwardsii*) were collected using crab traps (120 cm width base, 50 cm high, 30 cm width mouth, and 5 cm mesh size) left 12 h overnight with dead fish (*Genypterus blacodes*) as bait. Fishes and crabs were stored at −20 °C immediately after collection until further analysis at the laboratory.

Sampling and processing followed Levin and Currin (2012). Briefly, benthic organisms were identified to a low taxonomic level, and muscle or body wall tissues were extracted and rinsed with distilled water. All samples (consumers and food sources) were dried at 40°-60 °C for 24–48 h and stored in a desiccator. Tissue samples and macroalgae were ground to a fine powder in an Agatha mortar and pestle, dry weight was estimated using an electronic balance with a precision of 0.1 mg, and were then stored in numbered vials in a desiccator until sent to analysis. To remove carbonate content in samples (SOM, SPOM, macroalgae and tissues), an acidification treatment was performed (5%–10% solution of 1 N HCl). In samples with no calcified tissues (e.g., muscle tissue of fishes and crabs), no HCl treatment was needed (Jacob et al., 2005; Carabel et al., 2006; Levin and Currin, 2012). Since lipids are depleted in ^{13}C (DeNiro and Epstein, 1978) and are thought to introduce bias into ^{13}C stable isotope analyses, mathematical normalization for aquatic animals was applied following the linear equation: $\delta^{13}C_{lip\text{-}corr} = \delta^{13}C - 3.32 + 0.99 \times C{:}N$ (Post et al., 2007). Stable isotopes analysis ($\delta^{13}C$, $\delta^{15}N$), including the determination of carbon and nitrogen concentrations, were carried out in the Laboratory of Applied Stable Biogeochemistry and Isotopes (LABASI) of the *Pontificia Universidad Católica de Chile* using an Isotope Ratio Mass Spectrometer, IMRS, Thermo Delta Advantage coupled to an Elemental Flash EA2000 analyzer. The associated isotopic error was 0.16‰ and 0.30‰ for nitrogen and carbon, respectively. The isotopic values were expressed in conventional δ notation (‰) with respect to conventional standard:

$$\delta X\text{sample} = \left[\left(R_{sample}/R_{standard}\right) - 1\right] \times 1000$$

where X is ^{13}C or ^{15}N, and R is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. All results are reported with respect to the VPDB (Vienna PeeDee Belemnite) for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$.

The trophic position (TP) of each sample was calculated following the equation detailed by Vander Zanden and Rasmussen (1999):

$$TP_{consumer} = 2 + \left(\delta^{15}N_{consumer} - \delta^{15}N_{base}\right)/3.4$$

where $TP_{consumer}$ is the consumer trophic position of a particular species/group, $\delta^{15}N_{consumer}$ is the measured $\delta^{15}N$ value of the consumer analyzed, and $\delta^{15}N_{base}$ is the nitrogen baseline value. Because $\delta^{15}N$

values of primary food sources are influenced by spatial and temporal parameters (Post, 2002; Iken et al., 2010), our $\delta^{15}N_{base}$ were calculated used $\delta^{15}N$ values belonging to *Aulacomya atra* (n = 51), a suspensivore bivalve and primary consumer of phytoplankton (Montero et al., 2021), obtaining an average value of $\delta^{15}N$ 9.1 ± 0.7‰. The constant 2 corresponds to the level of primary consumer of the food web (Vander Zanden and Rasmussen, 1999), and the value of 3.4‰ was assumed as the average $\delta^{15}N$ enrichment per trophic level (Post, 2002).

Normality was tested with the Shapiro-Wilk test, with significance levels set at 5%. For multiple comparisons, the data sets did not meet the normality requirements, so the Kruskal-Wallis test with a post-hoc test was used (Zar, 1999). Differences in isotopic signatures among stations along the fjord (inner, middle and outer positions; Table 1) were evaluated using a one-way permutational multivariate variance analysis (PERMANOVA; Anderson, 2001) using statistical package Past 4.03b (Hammer et al., 2001). A hierarchical cluster analysis was applied to search for associations among the taxa/species and isotopic signatures. Mean values of $\delta^{13}C$ and $\delta^{15}N$ of each species were used to perform a resemblance measure with Euclidean distance. Cluster analysis was performed with the software PRIMER V6.1.6 (Clarke et al., 2008).

Stable isotope analyses were performed using the Bayesian mixing model SIAR (Stable Isotope Analysis in R; Parnell et al., 2010) and SIMMR (Stable Isotope Mixing Model; Parnell, 2016) from the package SIAR. Both were used to estimate the relative contribution of each food source to the study site and to consumer's diets. This analysis assumes that the system is being fed by macroalgae, SPOM and surface sediment. The trophic enrichment factor (TEF) was 1 ± 0.4‰ for $\delta^{13}C$, and 3.4 ± 1‰ for $\delta^{15}N$ (Vander Zanden and Rasmussen, 1999; Post, 2002). For consumers and functional groups based on the feeding guild for each taxon, lipid-corrected $\delta^{13}C$ values were used (Post et al., 2007). Groups with limited data ($n < 5$) were not included in the stable isotope analysis. Isotopic niche was studied using the SIBER package (Stable Isotope Bayesian Ellipses), and isotope niche width estimated and compared across trophic groups using standard ellipse areas (SEA), corrected for small sample sizes (SEAc) and total area (TA), following Jackson et al. (2011). Additionally, isotopic niche overlap among trophic groups was determined by the overlap area using SIBER at 95% confidence. Niche width overlap of a given trophic group within the niche width area of another trophic group was expressed as percentage. All analyses, simulations and plots were performed in RStudio version 4.0.5 (R Core Team, 2021).

Isotopic compositions among food sources showed significant differences (Kruskal-Wallis test; $\delta^{13}C$: $\chi 2 = 38.5$, df = 4, $p < 0.0001$; $\delta^{15}N$: $\chi 2 = 17.0$ df = 4, $p < 0.005$). SOM and SPOM presented significantly lower $\delta^{13}C$ values than macroalgae (paired *t*-test; $p < 0.005$ and $p < 0.05$, respectively). For SOM, the mean of $\delta^{13}C$ values varied from −28.39 ± 0.06‰ to −25.05 ± 2.98‰ along the fjord; and, for SPOM, −25.11 ± 2.66‰ (Fig. 2 and Supplementary data). Macroalgae presented high $\delta^{13}C$ values in relation to other food sources, from −17.34 ± 1.03‰ (brown macroalgae) to −13.71 ± 1.98‰ (red macroalgae). The mean $\delta^{15}N$ values of food sources ranged from 3.96 ± 1.69‰ (SOM) to 7.66 ± 0.72‰ (green macroalgae) (Fig. 2 and Supplementary data). SOM presented significantly lower $\delta^{15}N$ values than green and red macroalgae (paired *t*-test; $p < 0.05$), though no significant differences were detected between brown macroalgae and SPOM (paired *t*-test; $p > 0.05$). SPOM exhibited low $\delta^{13}C$ values and high $\delta^{15}N$ values (6.02 ± 2.22‰), but no significant differences in $\delta^{15}N$ were obtained between SPOM and other food sources (paired *t*-test; $p > 0.05$).

A total of 349 consumers were sampled in the *Puyuhuapi* fjord, covering a wide range of faunal groups, distributed across 26 species of marine invertebrates and 3 species of fishes. The mollusks (n = 9), porifera (n = 6), and echinoderms (n = 4) were the main phyla analyzed. The consumers exhibited significant differences for $\delta^{13}C$ and $\delta^{15}N$ values (Kruskal-Wallis test; $\delta^{13}C$: $\chi^2 = 144.9$, df = 28, $p < 0.0001$; $\delta^{15}N$: $\chi^2 = 184.9$, df = 28, $p < 0.0001$). The values of $\delta^{13}C$ for consumers fluctuated between −22.18‰ (*C. chilensis*) and −12.85‰ (*Tegula atra*), while values of $\delta^{15}N$ fluctuated between 6.20‰ (*Crepipatella dilatata*) and 16.73‰ (*I. comauensis*) (Supplementary data). Considering all consumers together without separating by trophic or taxonomic group, no significant differences in the $\delta^{13}C$ and $\delta^{15}N$ compositions were found along the fjord (inner, middle and outer sections) (PERMANOVA one-way test; $\delta^{13}C$: pseudo-F = 2.50, $p > 0.05$; $\delta^{15}N$: pseudo-F = 0.20, $p > 0.05$).

Isotopic compositions of functional guilds showed four groups: (1) Suspension feeders (SS: 17 species), (2) Carnivores (CA: 7 species), (3) Grazers (GR: 4 species), and (4) Depositivores (DD: 1 species) (Fig. 2).

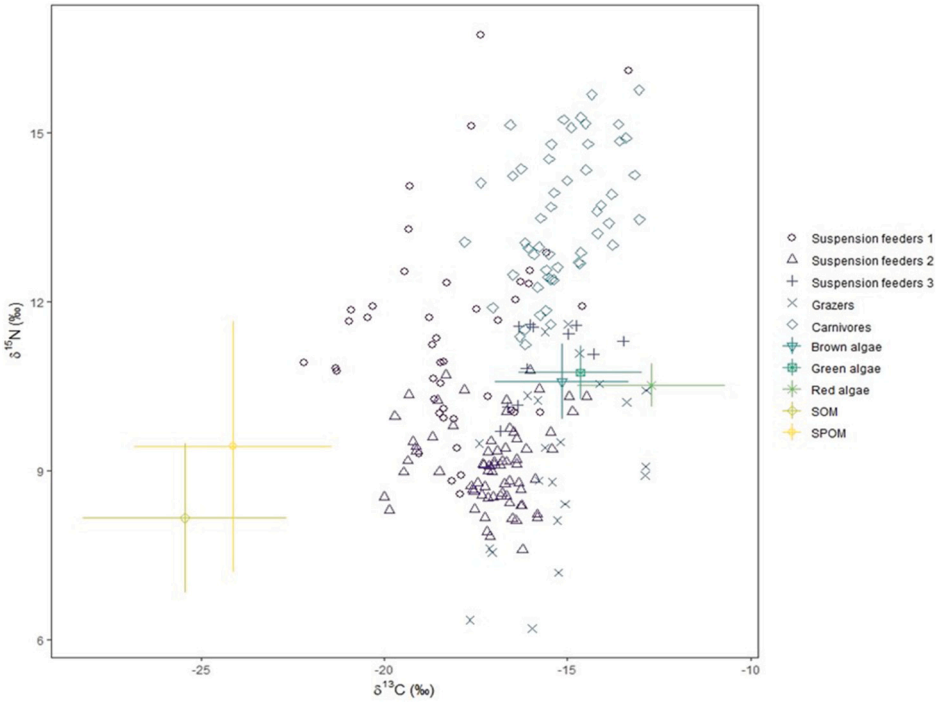

Fig. 2. Biplot of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) ratios (mean ± standard deviation) for different trophic groups and their potential food sources in the *Puyuhuapi* fjord. Bivariate graph constructed using Bayesian SIAR mixing model (Parnell et al., 2010). Trophic groups = SS1: Suspension feeders, SS2: Suspension feeders, SS3: Suspension feeders, GR: Grazers, CA: Carnivores. Sources = Brown macroalgae, Green macroalgae, Red macroalgae, SOM: Sediment organic matter and SPOM: Suspended particulate organic matter. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The SS group exhibited a wide distribution of isotopic composition, with mean values of $\delta^{13}C$ between −21.32 ± 0.02‰ (*C. chilensis*) and −14.71 ± 1.49‰ (*Magellania venosa*); and of $\delta^{15}N$, between 8.32 ± 0.21‰ (*Mytilus chilensis*) and 15.98 ± 0.82‰ (*I. comauensis*). The CA group presented a narrower amplitude, with $\delta^{15}N$ between 11.49 ± 0.26‰ (*Acanthina monodon*) and 15.46 ± 0.43‰ (*Prolatilus jugularis)*; and $\delta^{13}C$, between −17.24 ± 0.17‰ (*Cosmasterias lurida*) and −13.07 ± 0.32‰ (*P. jugularis*). The GR group was similar to the CA group in $\delta^{13}C$, with values between −17.28 ± 0.33‰ (*C. dilatata*) and −13.12 ± 0.37‰ (*T. atra*); and lower values of $\delta^{15}N$ in relation to the other trophic groups, between 6.69 ± 0.70‰ (*C. dilatata*) and 11.38 ± 0.27‰ (*Arbacia dufresnii*). The DD group, represented by *Munida gregaria*, had mean $\delta^{13}C$ values of −16.94 ± 0.02‰, and $\delta^{15}N$ values of 11.05 ± 0.17‰ (Supplementary data).

Cluster analysis showed four groups (Fig. 3): (1) mainly porifera and bivalvia, (2) mainly suspension feeders, (3) *C. chilensis* and Ascidiacea, and (4) carnivores (invertebrates and fishes) and *I. comauensis.* In order to examine isotopic data more closely, suspension feeder groups were further subdivided in three subgroups: (1) suspension feeders (SS1) composed of porifera and cnidaria (*Axinella crinita*, *C. chilensis*, *Tedania* sp., Porifera sp. 1, Porifera sp. 2, Porifera sp. 3, *I. comauensis* and *Thouarella* sp.); (2) suspension feeders (SS2), composed of the bivalvia group (*M. chilensis*, *Ameghinomya antiqua, Aulacomya atra*, *Gari solida* and *Zygochlamys patagonica)*; and (3) suspension feeders (SS3) composed of Holothuroidea and Brachiopoda (*Heterocucumis godeffroyi*, *Pentactella leonina* and *Magellania venosa*). These suspension feeder subgroups exhibited significant differences for $\delta^{13}C$ and $\delta^{15}N$ values (Kruskal-Wallis test; $\delta^{13}C$: $\chi^2 = 28$, df = 2, $p < 0.0001$; $\delta^{15}N$: $\chi^2 = 65$, df = 2, $p < 0.0001$).

The distribution of isotopic signatures of consumers falls within the polygon defined by macroalgae, SPOM and SOM. Our SIAR output indicated high $\delta^{15}N$ values (>12‰) for most SS1 (Fig. 2). The $\delta^{13}C$ values of SPOM and SOM were low with respect to macroalgae, while SS1 values fell in the rank of SPOM (−25 ± 2‰). Low $\delta^{15}N$ values were associated with GR and SS2 groups, and high $\delta^{15}N$ values were registered for CA and SS1 (Fig. 2). In general, potential food source contributions to consumers indicated that macroalgae predominated over other sources (SOM and SPOM) (Fig. 4). The contribution of red macroalgae was higher in grazers (47.5 ± 19.5%). However, green macroalgae exhibited a major contribution in CA (40.3 ± 19.9%) and SS3 (28.4 ± 18.2%), and brown macroalgae presented a higher contribution in SS1 (26.5 ± 18.5%). The SS1 and SS2 exhibited the highest proportions of SPOM as food source (SS1, 22.5 ± 7.6%; SS2, 13.3 ± 5.9%), indicating an important role of pelagic food sources in the diet of these groups, unlike other trophic groups with low SPOM contributions (mean < 6%) (Fig. 4). The contribution of SOM to the diet of the different trophic groups was generally low (mean < 5% in GR, SS3 and CA), but contributed in a higher percentage in two suspension feeder groups (SS1, 8.7 ± 5.9%; SS2, 7.8 ± 4.1%) (Fig. 4).

The relative trophic positions (TP) estimated for benthic consumers in the *Puyuhuapi* fjord was similar to those in other fjords (Zapata-Hernández et al., 2016; Cari et al., 2020), except for some suspension feeders. In general, an important number of species were primary consumers (TP = 2; n = 17), represented mostly by suspension feeders. Secondary consumers were represented by carnivores (TP = 3; n = 6) and an encrusting coral with suspensivore feeding mode (TP = 3.7 ± 0.5 for *I. comauensis*). In addition, other benthic species presented a trophic position under 2, mostly suspension feeders and grazers (n = 5) (Supplementary data).

Analyses of isotopic niche width for trophic groups included the standard ellipse area (SEAc) and overlap (95% Bayesian ellipses) among functional groups (Fig. 5). The SEAc for the SS1 group presented the highest isotopic niche width (SEAc = 10.22), followed by grazers (SEAc = 6.32), carnivores (SEAc = 3.92), and the SS2 and SS3 groups (SEAc = 2.71 and SEAc = 2.21, respectively) (Table 2). All functional groups showed overlap, with higher areas of niche overlap for suspension feeder and grazer groups. The SS1 group had high overlap percentages with the SS3 (90%), SS2 (86%), and CA (70%) groups; and the grazer group, with SS3 (89%) and SS2 (75%) (Table 2). Despite presenting the largest standard ellipse area (SEAc), SS1 showed less niche overlap in comparison to the other functional groups; the highest percentage of overlap (30%) was with the carnivore group. The SS3 group exhibited moderate to high overlap (between 36 and 90%) with all functional groups. The lowest overlap (2–3%) was observed between carnivore and the SS2 group (Fig. 5; Table 2).

Our results showed low $\delta^{13}C$ values in SOM (−28‰) and SPOM (−25‰), indicating an important contribution of tOM to the food web. These isotopic values – consistent with previous studies for the Patagonian fjords (Sepulveda et al., 2011; Silva et al., 2011; González et al., 2019; Cari et al., 2020) – indicate that river and runoff are a high OM

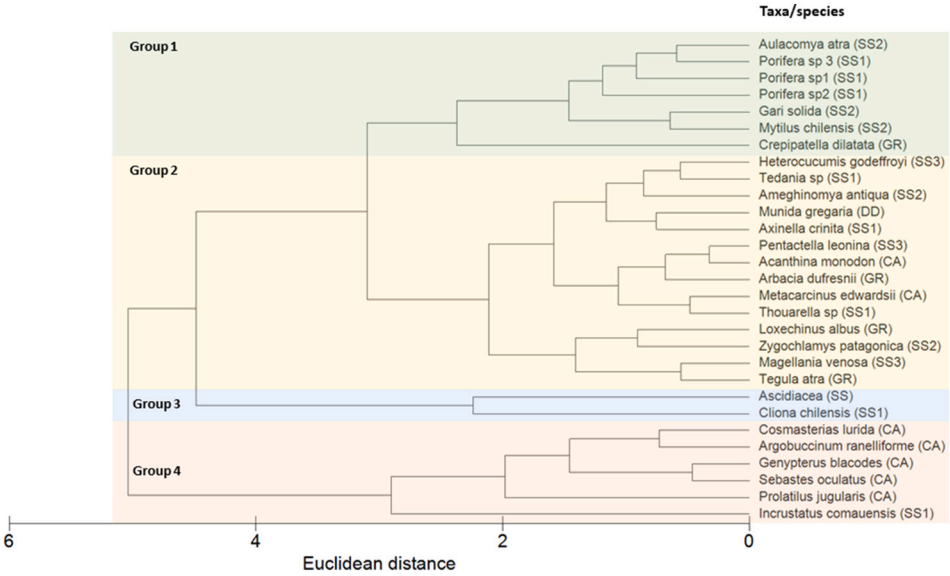

Fig. 3. Dendrogram based on mean $\delta^{13}C$ and $\delta^{15}N$ values of benthic community species in the *Puyuhuapi* fjord. Groups were classified using hierarchical cluster analysis, and different colors indicate the four main clusters. Trophic groups = SS: Suspension feeders, SS1, SS2 and SS3: Subgroups of Suspension feeders; GR: Grazers; CA: Carnivores.

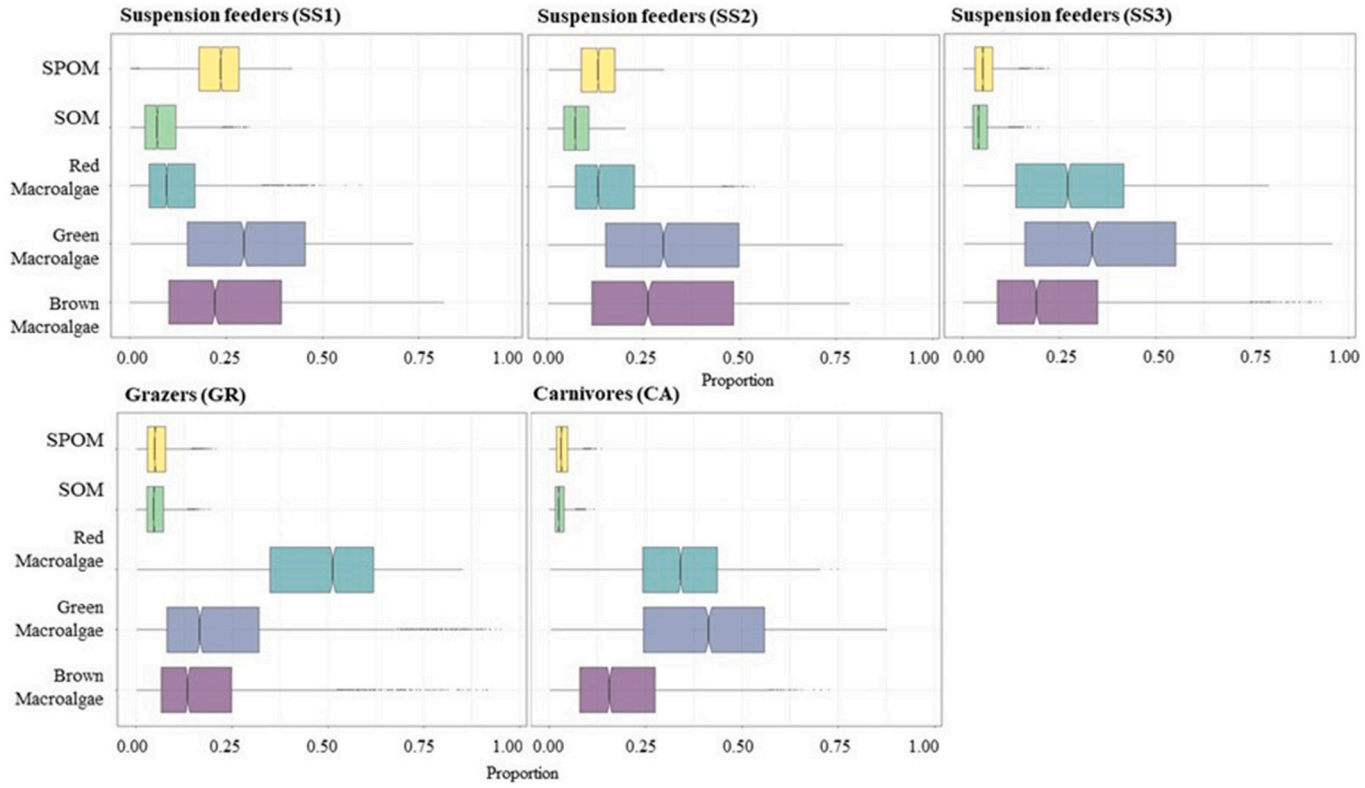

Fig. 4. Relative contribution of primary food sources to trophic group diets in the *Puyuhuapi* fjord, according to the SIAR mixing model (SIMMR). Boxplots represent 95% confidence intervals of each food source assimilated in the diet of each trophic group. The box is built around the 25th and 75th quartiles, thereby representing 50% of the solutions, and the centerline in the box indicates the median of all solutions.

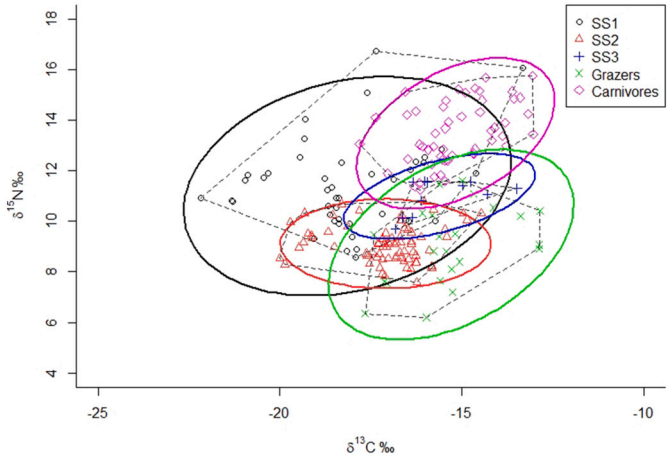

Fig. 5. Isotopic niche width of species evaluated in the *Puyuhuapi* Fjord. Solid lines enclose the standard ellipse area (SEAc), representing the isotopic niche of consumers. Dotted lines (convex hulls) representing the total niche width of the consumer. Trophic groups = SS1: Suspension feeders, SS2: Suspension feeders, SS3: Suspension feeders, GR: Grazers, CA: Carnivores.

subsidy (e.g., emergent vascular terrestrial woody plants and freshwater phytoplankton) to the benthic ecosystem (66–96%) particularly in the continental fjord environments such as the *Puyuhuapi* fjord (Silva et al., 2011; Sepulveda et al., 2011). The literature has reported carbon isotopic values – utilized as a proxy to identify OM sources – for terrestrial plants ($\delta^{13}C$; −31‰ to −26‰; Hedges et al., 1988; Le Blanc et al., 1989) and marine phytoplankton ($\delta^{13}C$; −22‰ to −18‰; Goericke and Fry, 1994). Based on Bayesian mixing model, our isotopic results for food sources showed a range of $\delta^{13}C$ values (−21‰ to −19‰) indicative of

Table 2
Trophic structure metrics for benthic community functional groups in *Puyuhuapi* fjord. TA: total area, SEA: standard ellipse area, SEAc: corrected standard ellipse area. The percentage of overlap (%) of each functional group in the rows is observed in 95% of total functional group standard ellipse area in the columns, and vice-versa.

	Functional groups				
	Suspension feeders			Grazers	Carnivores
	SS1	SS2	SS3	GR	CA
Metrics					
TA (‰^{2})	40.73	12.45	2.96	17.32	14.36
SEA (‰^{2})	9.98	2.69	1.99	6.04	3.84
SEAc (‰^{2})	10.22	2.72	2.21	6.32	3.92
% Overlap area					
SS1	100	23	19	27	29
SS2	86	100	29	75	3
SS3	90	36	100	89	46
GR	44	32	31	100	15
CA	70	2	26	24	100

important contributions to the benthic food web from marine phytoplankton OM (Sepulveda et al., 2011; Zapata-Hernández et al., 2016) particularly as a food source for SS2 bivalves, notable for their selective preferences for small seston associated with marine phytoplankton (Dubois et al., 2007; Mayr et al., 2011; Cranford, 2019; Montero et al., 2021).

Fjords in Northern Patagonia have previously registered low $\delta^{15}N$ values in water columns and sediments (Silva and Prego, 2002; Silva et al., 2011; Rebolledo et al., 2019), as well as in benthic macroalgae (Mayr et al., 2011). In general, nutrient inputs in fjord waters enriched with nitrate and orthophosphate are derived from oceanic Sub Antarctic Water masses (SAAW) (Silva and Palma, 2008). In our study area, the

isotopic signatures of food sources indicate that nutrients for primary productivity (macroalgae and SPOM) depend on a mixture of marine and terrestrial sources. However, an additional contribution of dissolved organic and inorganic nutrients from salmon farm waste may be enriching the water column.

In previous mass balance models (Mente et al., 2006), salmon farming activity may contribute up to 50% of the nitrogen and 28% of the phosphorus supplied from food as dissolved waste. Since our results showed an alteration of the $\delta^{13}C/\delta^{15}N$ ratio stoichiometry in *C. chilensis* and *I. comauensis*, it is likely that dissolved nitrogen contributions from these sources may be incorporated by suspension feeders species in the *Puyuhuapi* fjord. This is also supported by the high trophic overlap among groups - and thus higher trophic redundancy - in the benthic community (Włodarska-Kowalczuk et al., 2005; Quiroga et al., 2012; Cari et al., 2020). Indeed, as well as higher SEAc values, isotopic compositions exhibited depleted carbon and enriched nitrogen signals for SS1. Additionally, while low $\delta^{13}C$ values in *C. chilensis* are associated with significant tOM contributions, the higher $\delta^{15}N$ values appear to be related to nitrogen compounds derived from salmon farms; however, here holobiont-microbiome interactions may also be involved (Kahn et al., 2018). Briefly, sponges feed on bacteria (photosynthetic or heterotrophic), and therefore may have different isotopic signatures by assimilating dissolved organic carbon and nitrogen directly via microbial consortia (Mohamed et al., 2008; Rix et al., 2020; Campana et al., 2021). Thus holobiont-microbiome interactions can produce changes in the isotopic niche width and trophic structure in suspension-feeder benthic communities, and indeed, sponges and cnidarians host diverse microbial communities which may also influence functional OM processing and ecological stoichiometry (e.g., Apprill, 2017; Pita et al., 2018; Cleary et al., 2020; Dittami et al., 2021; Maldonado et al., 2021).

In general, octocorals may filter a wide rank of particles, incorporating bacteria, diatoms, dinoflagellates or microzooplankton (Lewis, 1982; Anthony, 1999; Rossi et al., 2004; Orejas et al., 2003). In our study area, high $\delta^{15}N$ values - similar to those registered in large-predator species such as asteroidea, crustaceans, and gastropods - were found in an octocoral (*I. comauensis*) ($\delta^{15}N$ 15.98 ± 0.82‰). In fact, suspension-feeders such as sponges, cnidarians and tunicates, which relied on naturally $\delta^{15}N$-depleted sources, have been registered as $\delta^{15}N$-enriched near the fish farm (Lojen et al., 2005; Dolenec et al., 2007; Wai et al., 2011; Callier et al., 2013). Suspension feeder such as *C. chilensis* and *I. comauensis* may feed on dissolved OM and therefore assimilate an important fraction of dissolved nitrogen (Anthony, 1999; Pita et al., 2018; Rix et al., 2020; Maldonado et al., 2021). Moreover, SS1 group also exhibited trophic niche overlap with the carnivore group, suggesting that it may be involved in nitrogen compound assimilation from salmon fish waste as has been reported by Woodcock et al. (2018). Additionally, cluster analysis grouped *I. comaunesis* with carnivores, and it had the highest trophic position of all the taxa evaluated in this study, exceeding estimated values for fishes, which indicates that enrichment from one trophic level to the next (i.e., 3.4‰ for $\delta^{15}N$; Vander Zanden and Rasmussen, 1999; Post, 2002), is not the only factor controlling the high $\delta^{15}N$ values in this octocoral. Therefore, it is important to understand the ways of incorporation of the OM, given the different responses of the suspension feeders in the particulate and dissolved nitrogen assimilation (Wai et al., 2011; Callier et al., 2013).

On the other hand, bivalve mollusks (SS2) showed isotopic signals probably related to phytoplankton and/or resuspended detritus of macroalgae (Bearham et al., 2020). Montero et al. (2021) analyzed tissues of bivalve *A. atra* and SPOM in the study area and found that this bivalve seems to feed on either autochthonous (bacterial and microplanktonic community) or allochthonous (salmon food pellet) OM. Benthic grazers also had a predominantly macroalgae diet, associated with high $\delta^{13}C$ values and the lowest $\delta^{15}N$ values compared to SS and CA groups. However, this group (GR) showed a major isotopic niche width, which indicates a mixture of food sources associated with opportunistic behavior (Andrade and Brey, 2014; Andrade et al., 2016). Finally, the CA group presented a SEAc lower than those of the GR and SS groups, an isotopic niche associated with high $\delta^{15}N$, within the range reported for predators (i.e., asteroidea, crustaceans and fishes) in other areas of Chilean Patagonia (Mayr et al., 2011; Andrade et al., 2016; Zapata-Hernández et al., 2016; Bernal Bajo et al., 2020; Cari et al., 2020).

In fjord environments, the quality and quantity of food sources for the benthos are related to complex environmental and biological interactions (Alurralde et al., 2020; Cari et al., 2020; Montero et al., 2021) and the resulting trophic interactions among benthic community species (Sokolowski et al., 2012). Therefore, our understanding of OM exchange from different origins (autochthonous and allochthonous) – as well as temporal and spatial variability of OM input - is key for a more detailed predictions about secondary production, diversity patterns, and food web dynamics (Jacob et al., 2014; Andrade et al., 2016; Quiroga et al., 2016; Montero et al., 2017a, 2021). This study showed that isotopic niche width of benthic species in the *Puyuhuapi* fjord was highly variable, probably in response to food supply derived from marine and terrestrial OM. In general, an important fraction of feed pellets and fish faeces can be assimilated by epibenthic fauna and infauna located close to the farms (Callier et al., 2013; Woodcock et al., 2018). In our study area, the isotopic signatures of feed pellets ($\delta^{15}N = 8.3 \pm 2.1$‰) and fish faeces ($\delta^{15}N = 10.6 \pm 0.3$‰) were determined by Sepulveda et al. (2011), exhibiting significant differences from terrestrial organic matter from riverine sediment ($\delta^{15}N$ ranged from −1.1 to 2.4‰). In addition, isotopic signatures for *A. atra* showed that bivalves preferentially exploit food resources with signals consistent with autochthonous marine OM, although isotopic composition of the salmon food pellets used in feeding experiments showed overlaps with marine phytoplankton signal in SPOM samples (Montero et al., 2021). These results suggest that *C. chilensis* and *I. comauensis* may have assimilated an important fraction of dissolved nitrogen probably derived from salmon farming waste, exhibiting enriched values of $\delta^{15}N$. However, the contribution of nitrogen to the food web, shaped a benthic community with higher trophic redundancy, with different isotopic signals for the trophic group of suspension feeders, and showed sponges and cold-water corals (SS1) $\delta^{15}N$-enriched. Suspension feeders such as sponges and tunicates appear to be adequate tracers of marine aquaculture nitrogen enriched waste independent of the environmental variability of the system (Dolenec et al., 2007; Callier et al., 2013, Woodcock et al., 2018).

The Chilean Patagonia hosts one of the most extensive fjord systems in the world, providing important ecosystem services; however, they are vulnerable to environmental change. Suspension feeders such as *C. chilensis* and *I. comauensis* may be used as ecological indicators to evaluate stoichiometry imbalances. Environmental monitoring programs may make use of them to provide essential baseline information in identifying and trace different sources of organic pollution in fjord environments.

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Paula Ortiz: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Sampling activity, Data curation, Writing – original draft, Writing – review & editing, Visualization.

Eduardo Quiroga: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization.

Paulina Montero: Database, Writing – original draft, Writing – review & editing, Visualization.

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Federico Betti: Writing – original draft, Writing – review & editing, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

Supplementary Table S1. Results of isotopic composition ($\delta^{13}C$ and $\delta^{15}N$ ‰; mean ± SD), C/N ratios (mean ± SD) for primary sources in *Puyuhuapi* fjord, indicating number of samples (n), station, date for each source. SOM: Sediment organic matter, SPOM: Surface particulate organic matter.

Sources	n	Station	Fjord section	Date	$\delta^{15}N$ (‰) mean	SD	$\delta^{13}C$ (‰) mean	SD	C/N ratio mean	SD
SPOM	32	B4	Middle	March/2018 to February/2019	6.02	2.22	-25.11	2.66	7.14	1.70
SOM	3	B5	Inner	10/07/2018	3.96	1.69	-28.39	0.06	10.25	0.32
SOM	3	B5	Inner	20/02/2018	4.94	0.30	-28.20	0.26	9.87	0.66
SOM	8	B10-B11-B12-B13	Middle	19-20/01/2020	4.98	1.43	-25.05	2.98	11.32	3.60
Green macroalgae	3	B1	Outer	22/02/2018	7.66	0.72	-14.87	2.90	14.21	0.85
Green macroalgae	3	B9	Middle	10/12/2020	7.13	0.07	-16.18	0.35	10.57	0.13
Brown macroalgae	3	B7	Outer	21/02/2018	7.21	0.93	-17.34	1.03	18.27	5.19
Brown macroalgae	3	B9	Middle	10/12/2020	7.12	0.07	-14.38	0.34	19.00	1.47
Red macroalgae	7	B9	Middle	10/12/2020	7.11	0.36	-13.71	1.98	12.14	4.06

Supplementary Table S2. Results of isotopic composition ($\delta^{13}C$ and $\delta^{15}N$ ‰; mean per taxa ± SD), C/N ratios (mean per taxa ± SD) for consumers in *Puyuhuapi* fjord, indicating number of samples (n), station, date, feeding mode and trophic position for each taxa/species.

Taxa	Fjord section	Station	Date	n	$\delta^{15}N$ (‰) mean	sd	$\delta^{13}C$ (‰) mean	sd	$\delta^{13}C'$ (‰) mean	sd	C/N ratio mean	sd	Feeding Mode	Trophic position
Porifera														
Cliona chilensis	Inner	B8	16/07/2018	3	12.19	0.42	-20.81	0.83	-18.84	0.57	5.34	0.32	SS	2.9
Cliona chilensis	Middle	B3	13/07/2018	1	11.74	0.15	-23.10	0.17	-20.92	0.04	5.56	0.13	SS	2.8
Cliona chilensis	Inner	B5	21/02/2019	1	10.79	0.03	-24.55	0.75	-21.32	0.02	6.62	0.74	SS	2.5
Cliona chilensis	Outer	B1	04/03/2019	2	11.52	0.52	-23.97	1.08	-20.98	1.04	6.37	0.16	SS	2.7
Cliona chilensis	Middle	B9-B13	18-20/01/2020	6	10.66	0.77	-21.12	0.61	-18.63	0.23	5.86	0.46	SS	2.5
Cliona chilensis	Middle	B9	10/12/2020	2	10.58	0.06	-21.03	0.63	-18.56	0.15	5.85	0.48	SS	2.4
Tedania sp	Outer	B1	04/03/2019	3	12.40	0.12	-18.81	0.27	-16.11	0.14	6.08	0.13	SS	3.0
Tedania sp	Middle	B9	10/12/2020	3	10.13	0.15	-19.03	0.49	-16.48	0.72	5.94	0.39	SS	2.3
Axinella crinita	Middle	B9	18/01/2020	2	11.77	0.14	-21.11	0.32	-17.18	0.42	7.33	0.75	SS	2.8
Porifera sp 1	Middle	B13	20/01/2020	3	9.78	0.34	-21.24	1.26	-17.52	0.93	7.11	2.08	SS	2.2
Porifera sp 2	Middle	B13	20/01/2020	3	10.01	0.09	-20.31	0.36	-18.41	0.08	5.27	0.40	SS	2.3
Porifera sp 3	Middle	B13	20/01/2020	3	8.77	0.17	-19.32	0.22	-17.99	0.14	4.70	0.13	SS	1.9
Cnidaria														
Incrustatus comauensis	Outer	B1	11/07/2018	1	15.98	0.82	-17.82	1.87	-16.10	2.41	5.10	0.55	SS	4.0
Incrustatus comauensis	Inner	B5	21/02/2019	5	13.66	0.54	-21.88	0.03	-19.33	0.02	5.93	0.01	SS	3.4
Thouarella sp	Middle	B9	18/01/2020	3	12.27	0.52	-19.79	0.55	-15.52	0.90	7.67	0.46	SS	2.9
Mollusca														
Bivalvia														
Mytilus chilensis	Outer	B1	22/02/2018	18	8.32	0.21	-17.76	0.31	-16.31	0.47	4.81	0.32	SS	1.8
Mytilus chilensis	Outer	B6	22/02/2018	17	9.04	0.07	-17.25	0.14	-17.08	0.13	3.52	0.01	SS	2.0
Aulacomya atra	Middle	B2-B4	24/02/2018	7	8.60	0.87	-18.53	1.12	-16.98	0.45	4.92	1.35	SS	1.9
Aulacomya atra	Outer	B1-B7	21-22/02/2018	7	9.13	0.25	-17.77	0.76	-16.90	0.38	4.23	0.61	SS	2.0
Aulacomya atra	Inner	B5	20/02/2018	4	9.32	0.38	-18.20	0.82	-16.76	0.29	4.81	0.94	SS	2.1
Aulacomya atra	Middle	B2-B3-B4	10-11-15/07/2018	9	8.68	0.46	-18.99	0.53	-16.83	0.60	5.53	0.88	SS	1.9
Aulacomya atra	Outer	B1	11/07/2018	3	8.94	0.34	-18.06	0.16	-17.34	0.37	4.08	0.21	SS	2.0
Aulacomya atra	Inner	B5-B8	10-16/07/2018	6	8.71	0.43	-17.96	0.35	-16.40	0.25	4.93	0.45	SS	1.9
Aulacomya atra	Outer	B1	04/03/2019	3	10.46	0.22	-19.50	0.28	-18.22	0.37	4.65	0.40	SS	2.4
Aulacomya atra	Middle	B2-B3-B4	24/02/2019	9	9.22	0.64	-21.38	0.57	-19.39	0.45	5.36	0.86	SS	2.0
Aulacomya atra	Inner	B5	21/02/2019	3	9.63	0.14	-21.31	0.13	-18.68	0.54	6.01	0.42	SS	2.2
Gari solida	Middle	B5	20/02/2018	4	8.59	0.11	-17.91	0.50	-17.06	0.27	4.20	0.25	SS	1.9
Ameghinomya antiqua	Middle	B5	20/02/2018	5	10.14	0.52	-17.51	0.88	-16.27	0.36	4.61	0.64	SS	2.3
Zygochlamys patagonica	Outer	B1	04/03/2019	6	10.22	0.15	-14.74	0.26	-14.76	0.26	3.33	0.07	SS	2.3
Zygochlamys patagonica	Middle	B9	10/12/2020	6	9.41	0.27	-15.99	0.64	-15.85	0.71	3.50	0.08	SS	2.1
Gastropoda														
Crepipatella dilatata	Inner	B8	22/02/2018	8	7.17	0.71	-16.79	0.08	-17.28	0.33	2.85	0.39	GR	1.4
Crepipatella dilatata	Outer	B1	11/07/2018	3	6.69	0.70	-14.95	0.01	-15.61	0.51	2.69	0.52	GR	1.3
Tegula atra	Outer	B1-B7	21-22/02/2018	8	10.37	0.15	-15.12	0.97	-15.34	1.06	3.13	0.09	GR	2.4
Tegula atra	Inner	B5	20/02/2018	12	10.31	0.15	-12.83	0.34	-13.12	0.37	3.07	0.03	GR	2.4
Acanthina monodon	Inner	B5	20/02/2018	6	11.68	0.11	-15.92	0.26	-15.60	0.21	4.06	0.06	CA	2.8
Acanthina monodon	Outer	B6-B7	22/02/2018	12	11.49	0.26	-16.33	0.44	-16.05	0.32	3.64	0.12	CA	2.7
Argobuccinum ranelliforme	Outer	B1	22/02/2018	6	12.46	0.09	-15.53	0.17	-15.50	0.10	3.38	0.07	CA	3.0
Argobuccinum ranelliforme	Inner	B8	22/02/2018	2	12.96	0.02	-15.63	0.16	-15.93	0.21	3.05	0.05	CA	3.1
Argobuccinum ranelliforme	Middle	B2	11/07/2018	3	13.67	0.06	-14.85	0.94	-14.58	0.76	3.63	0.19	CA	3.4
Argobuccinum ranelliforme	Outer	B1	11/07/12018	3	14.56	0.55	-14.45	1.16	-14.11	1.09	3.69	0.07	CA	3.6
Arthropoda														
Decapoda														
Munida gregaria	Middle	B10	13/12/2020	31	11.05	0.17	-17.25	0.03	-16.94	0.02	3.67	0.03	DD	2.6
Metacarcinus edwardsii	Middle	B2	24/02/2018	3	12.43	0.25	-15.86	0.42	-15.55	0.38	3.67	0.03	CA	3.0
Metacarcinus edwardsii	Inner	B5	20/02/2018	2	12.94	0.14	-15.79	0.62	-15.84	0.46	3.30	0.16	CA	3.1
Metacarcinus edwardsii	Middle	B2	24/02/2018	3	12.69	0.25	-14.58	0.71	-14.64	0.69	3.29	0.11	CA	3.1
Metacarcinus edwardsii	Middle	B3-B10	12-13/12/2020	6	12.79	0.56	-15.73	1.14	-15.66	1.09	3.42	0.09	CA	3.1
Brachiopoda														
Magellania venosa	Outer	B1	04/03/2019	10	10.84	0.60	-14.93	1.82	-14.71	1.49	3.58	0.35	SS	2.5
Echinodermata														
Asteroidea														
Cosmasterias lurida	Inner	B5-B8	10/07/2018	5	14.41	0.26	-14.89	0.80	-14.53	0.87	3.72	0.23	CA	3.6
Cosmasterias lurida	Middle	B2	11/07/2018	3	14.10	1.04	-17.72	0.73	-17.24	0.64	3.83	0.32	CA	3.5
Cosmasterias lurida	Outer	B1	11/07/2018	2	14.29	0.10	-16.56	0.10	-16.38	0.17	3.54	0.07	CA	3.5
Cosmasterias lurida	Outer	B1	04/03/2019	3	13.58	0.28	-14.34	0.68	-13.57	0.46	4.14	0.22	CA	3.3
Echinoidea														
Loxechinus albus	Inner	B5	20-02-2018	3	8.44	0.34	-18.59	0.61	-15.25	0.18	6.46	0.47	GR	1.8
Loxechinus albus	Outer	B1-B7	21-22/02/2018	10	9.32	0.28	-17.95	0.98	-15.27	1.86	6.06	1.10	GR	2.1
Loxechinus albus	Middle	B12	19/01/2020	4	8.95	0.18	-16.80	1.70	-14.32	2.06	5.85	0.36	GR	2.0
Arbacia dufresnii	Middle	B12	19/01/2020	24	11.38	0.27	-16.72	0.55	-15.09	0.48	5.00	0.21	GR	2.7
Holothuroidea														
Heterocucumis godeffroyi	Inner	B8	22/02/2018	7	9.91	0.30	-17.10	0.10	-16.72	0.13	3.74	0.04	SS	2.3
Heterocucumis godeffroyi	Middle	B9	18/01/2020	10	11.26	0.40	-16.36	0.75	-15.80	0.73	3.91	0.32	SS	2.6
Pentactella leonina	Middle	B9	18/01/2020	10	11.57	0.02	-16.87	0.18	-15.58	0.71	4.65	0.54	SS	2.7
Chordata														
Ascidiacea														
Ascidiacea	Inner	B5	21/02/2019	1	9.64	0.16	-24.13	0.04	-21.23	0.12	6.28	0.16	SS	2.2
Osteichthyes														
Sebastes oculatus	Middle	B9	18/01/2020	3	15.36	0.28	-14.67	0.64	-14.65	0.40	3.38	0.24	CA	3.9
Genypterus blacodes	Middle	B3	12/12/2020	3	15.05	0.24	-14.85	0.39	-14.99	0.40	3.21	0.04	CA	3.8
Prolatilus jugularis	Middle	B13	20/01/2020	2	15.46	0.43	-13.07	0.32	-13.33	0.40	3.09	0.08	CA	3.9

Feeding Mode:
DD= Deposit feeder
SS= Suspension feeder
GR=Grazer
CA=Carnivore

Trophic position:
$TP_{consumer} = 2 + (\delta^{15}N_{consumer} - \delta^{15}N_{base})/3.4$

$\delta^{13}C'$: Lipid-corrected values

Chapter 3

Geographic patterns of soft-bottoms benthic communities in Chilean Patagonian fjords (47°S-54°S) - influence of environment stress on diversity and stable isotope signatures

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ABSTRACT

The benthic community in the Chilean Patagonia is as rich and highly diverse as the spatial variation of its habitats and food sources. Environmental stress from glacier melt and river discharge are known drivers of benthic community dynamics. Here we analyze longitudinal patterns of soft-bottoms benthic communities across the glacier-marine environments and how these habitats may respond to changes in the environmental conditions. To assess the relationships between environmental condition heterogeneity (marine and glacial habitats) and benthic biodiversity, this paper reviews biological (mega- and macroinfauna) and sedimentological dataset from multiple oceanographic campaigns in Chilean Patagonian fjords. Furthermore, we analyze isotopic compositions of epibenthic and macroinfaunal organisms (δ13C/δ15N and C/N) to determine POC and PON availability and organismal stoichiometric budgets within each benthic community. Our results showed that glaciomarine and glaciofluvial environmental stress negatively affected nutrient reservoirs and organic matter (OM) availability, and hence produced changes in benthic diversity and trophic structure throughout the fjords. These conditions shaped the benthic community where small-bodied polychaetes and opportunistic species dominated. Additionally, we found a strong relationship between OM and nutrient availability with faunal isotopic composition, but a

decoupling in C/N ratios between fauna and sediment OM, suggesting preferential organic carbon use under limited nitrogen in fjord sediments.

Keywords
glaciomarine and glaciofluvial environments, carbon and nitrogen stable isotopes, C/N ratios.

1. Introduction

The Patagonian fjords – glacial estuaries at extreme latitudes, characterized by elongated, deep channels, and sills – are considered Aquatic Critical Zones (ACZs), sensitive to biogeochemical imbalances related to organic carbon degradation, burial/sequestration, and budgets (Bianchi et al., 2020). Sedimentary processes in stratified, fjord-type circulation play an important role in biogeochemical cycles, supporting the thought these fjords are organic carbon sequestration hot spots (Faust and Knies, 2019; Włodarska-Kowalczuk et al., 2019a). Additionally, the unique biogeochemical and biological characteristics under which they incorporate, and process OM are influenced by local primary productivity dynamics and/or terrestrial organic matter (tOM) assimilation, controlling carbon sequestration efficiency (Bianchi et al., 2020).

The geographic benthic diversity patterns in Chilean Patagonian are highly important due to their high vulnerability to climate change, which may affect biogeochemical cycles and ecosystem functions. It is known that macrobenthic communities are highly sensitive to climate change in Arctic fjords (Włodarska-Kowalczuk et al., 2012; Renaud et al., 2019; McGovern et al., 2020). Fjord environments are influenced by harsh environmental conditions, negatively affecting benthic-pelagic coupling, carbon and nitrogen cycles, OM processing, respiration, and carbon sink balances (Barnes et al., 2018; 2020; Bianchi et al., 2020). And so, while it is known that marine benthic communities act as carbon sinks, the role of benthic organisms in fjord OM cycles has been largely neglected (Barnes et al., 2020). Furthermore, the loss of habitat or keystone species, which support high biodiversity (Häussermann and Försterra, 2009; Friedlander et al., 2021). However, studies integrating biogeochemical and ecological perspectives in marine ecosystems have been limited (Snelgrove et al., 2018). Therefore, the study of marine ecosystem food-web structures and transfer of OM from low trophic positions to upper trophic-level consumers provides fundamental insight into the relationship between biodiversity and ecosystems. Although

marine – terrestrial interactions in fjords are complex, macrobenthic communities play an important role in the distribution of OM. While most benthic realms tend to depend upon primary production, those in fjord ecosystems also receive OM from land and estuarine environments. The understanding of OM exchange from different sources is key to more detailed predictions about secondary production, diversity patterns, and food web dynamics, which are closely related to the temporal and spatial variability of freshwater dynamic from rivers and glaciers (Quiroga et al., 2016; Zapata-Hernández et al., 2016; Cari et al., 2020).

Different sources of OM shape benthic community trophic structure, contribute to complexity in benthic food-webs, modify local biodiversity patterns, and affect environmental conditions. In general, biodiversity is positively correlated with food chain length – i.e., energy is transferred through more trophic levels – while species-poor communities sustain a shorter food chain (Sokolowski et al., 2012; Cari et al., 2020). Marine macroalgae are also an important source of organic matter in coastal systems, contributing to the diet of grazers, suspension feeders and detritivores benthic fauna (Zapata-Hernández et al., 2016; Cari et al., 2020). In contrast, tOM is commonly sourced from river mouth dispersion plumes (e.g., Quiroga et al., 2016; Cari et al., 2020) and encourages high trophic redundancy (e.g., species with overlapping trophic niches) and stressed marine environments like glaciofluvial fjords (Cari et al., 2020). High sedimentation also occurs from periodic subglacial discharge, which increases as a function of distance from glaciers (Włodarska-Kowalczuk et al., 2012; Davies et al., 2020). Understanding this primary production (PP) and/or tOM exchange is key to making more detailed predictions about secondary productivity, diversity, and food web dynamics.

As described in Welti et al. (2018), a new approach for understanding organic matter cycling in aquatic ecosystems is ecological stoichiometry, defined as the balance of ecological interactions (Sterner and Elser, 2002; Van de Waal et al., 2018) as regards availability of OM (POC and PON reservoirs) and elemental compositions (i.e., $\delta^{13}C/\delta^{15}N$; C/N ratios) of benthic organisms. Stable isotopes are useful indicators of OM origin, and benthic organisms incorporate C-rich (e.g., carbohydrates, lipids) and N-rich (e.g., amino acids) organic compounds for metabolic processes, energy storage, and growth performance (Hunter et al., 2012) as determined by internal budgets, ontogeny stages, and physiology (Sterner and Elser, 2002). In this context, the fjords of the Chilean Patagonia (42°S – 56°S)

present a unique research opportunity, especially for their high sensitivity to environmental changes and positive high rates of glacial recession (Davies et al., 2020). Indeed, as one of the most extensive fjord regions in the world – with nearly 84,000 Km of coastline (Silva and Palma, 2008) – there is wide spatial and temporal hydrographic variability due to local influences from winds, tides, and continental freshwaters. In this proglacial coastal dynamic, it is important to measure to what extent sedimentation drives benthic habitats.

Although there have been few studies on Patagonian glaciofluvial and glaciomarine environments, analogous descriptions for deep Arctic fjords found that suspended sediment material and water mass dynamics shaped homogenous and very stable benthic communities (Jordà-Molina et al., 2019). Studies on benthic communities in the Southern Patagonian (47°-54°S) have generally been limited to the Martinez-Baker fjord system (MBFs), the Strait of Magellan, the Messier and Beagle Channels, and off of the Taitao Peninsula (e.g., Arntz and Rios, 1999; Häussermann and Försterra, 2009; Rios et al., 2013), usually under the auspice of international oceanographic expeditions, e.g., Interactions Between the Magellan Region and the Antarctic (IBMANT) or the International South-East Pacific Investigation of Reducing Environments (INSPIRE). In addressing this lack of data on benthic community species distribution, trophic structure, and biodiversity in fjords, the CIMAR program – organized by the Chilean National Oceanographic Committee (Silva and Palma, 2008) – has provided unparalleled access to remote areas of the Chilean Patagonia (Rios et al., 2005, 2013). However, extensive areas remain unexplored, and benthic invertebrate diversity inventories for the region continue to be inadequate (Arntz and Rios, 1999; Häussermann and Försterra, 2009). We hypothesize that environmental stress associate to glaciofluvial and glaciomarine systems will define the diversity patterns and organismal stoichiometry of benthic along the coast and between the open coastal zone and adjacent fjords. As such, this paper presents quantitative research data in from the Martinez-Baker fjord (MBF) complex (47°S) to the Magellan Strait (54°S) – encompassing the South Patagonian Icefield (SPI) region – in order to: (i) characterize the diversity, feeding modes, and relative abundances of epibenthic communities and macroinfauna in the Chilean Patagonian fjords associated with the SPI; and (ii) determine the effect of high sedimentation rates and terrestrial OM input on organismal isotopic composition and C/N ratios in a Chilean Patagonia fjord heavily influenced by a glaciofluvial environment. This study involves one of the most extensive

fjord ecosystems in the southern hemisphere (Bianchi et al., 2020); therefore, our comprehensive spatial analysis constitutes a regional assessment of ecological patterns which may serve as a baseline to detect changes in the biodiversity and ecological stoichiometry in Patagonian benthic communities under the different scenario of climate change.

2. Methods

2.1. Study Area

The geography of the Chilean Patagonia coastal system (41°–55°S) is highly complex, with channels, islands, and fjords ranging from 500 to 2000 m deep. There are three main glacial fields nestled in these mountains: The North Patagonian Icefield (NPI), from 46° to 47°S; the SPI, between 48° and 52°S; and the Darwin Mountain Range, 54° and 55°s. Breaking up at the fjord heads, icebergs from the glaciers descending toward the coastline dilute seawater. Due to glacial valley stages of development, many fjord basin bottoms are composed of glacial sediments (Silva and Palma, 2008; Moffat et al., 2018).

There is scarce information on the complex interactions between ocean thermal forcing and glacier freshwater discharge from studies on hydrography and submarine landforms in fjords surrounding ice fields (Dowdeswell and Vásquez, 2013; Rios et al., 2016; Moffat et al., 2018). Between SPI glaciers and the Pacific Ocean, the complex network of fjords creates gradients of heat content and deep water-glacier contact (Moffat et al., 2018). The Patagonian fjords are further supplied with sediment inputs from turbid meltwater produced by ice-surface ablation and snowmelt. Although this is seasonal – maximized in the summer, when subglacial discharge is at its peak – distribution of debris over the adjacent fjord systems results in laminated fine-grained sediments (Dowdeswell and Vásquez, 2013).

In terms of water masses, the Chilean Patagonia has strong river runoff and glacier freshwater lateral advection (river-ocean) that controls hydrographic characteristics and primary productivity patterns. This mix of water masses influences mixing/stability processes, which are important factors for vertical distribution of chlorophyll-a in glacier environments (Rios et al., 2016). The largest rivers catchment flows are that of Aysén (300 $m^3 s^{-1}$), Baker (1100 $m^3 s^{-1}$), Pascua (800 $m^3 s^{-1}$) and Bravo (120 $m^3 s^{-1}$). Rainfall varies from 1000 to 7000 mm/year on the western side of the Andes, highest just north of the Magellan

Strait[1]. In general, upper water columns are characterized by low nitrate and phosphate freshwater discharges from continental runoff, rivers, and glacial tributaries, before contrasting with high silicic acid concentrations (Quiroga et al., 2016; Moffat et al., 2018). Below the pycnocline, oceanic sub-Antarctic waters (SAW) increase nutrient concentrations, resulting in strong vertical and horizontal hydrographic gradients that fluctuate depending on the volume and dominant source of freshwater input. As a result, there is a highly stratified estuarine environment with marked longitudinal gradients of suspended particulate material.

2.2. Macroinfauna sampling

To characterize diversity patterns and ecological processes in the MBF, samples were obtained during seasonal campaigns onboard the *RV* Sur Austral (University of Concepción) in March 2014 and December 2015. Water samples for POC analysis were collected with a bottle-rosette system at 11 stations between the Baker River mouth and the *Golfo de Penas* on the Pacific Ocean shelf. For nutrient analyses (nitrate, orthophosphate, and silicic acid), 50 mL samples were collected at selected depths (1, 5, 10, 25, 50, 75 m, and bottom), stored at -20 °C in acid-cleaned high-density polyethylene bottles, and analyzed with a nutrient autoanalyzer (Technicon) following Atlas et al. (1971). Independent replicates of sediment samples were collected using a gravity corer (50 mm internal diameter, 1000 mm length) and modified van Veen grab (0.052 m^2). Surface sediment samples were kept frozen (-20°C) prior to analyses of sediment organic matter (SOM) and stable carbon and nitrogen isotopes (Figure 1, Supporting information Table S1). To describe macroinfaunal diversity patterns, we collected 2-6 replicate with grab sampler, excluding faunal groups not quantitatively sampleable by this method, such as nematodes and foraminifera (Quiroga et al., 2016). Sediment samples were sieved through a 500-μm mesh screen, and the biological material was fixed in a 10% buffered formaldehyde-seawater solution.

2.3. Epibenthic fauna sampling

To describe regional diversity patterns, megafaunal benthic communities were sampled using a modified Agassiz trawl (AGT: beam, 1.2 m width x 0.5 m high; mesh size, 1 cm internal,

4 cm external) on board the *RV Cabo de Hornos* as part of CIMAR Fjord expeditions: (i) oceanographic CIMAR fjord cruise No. 20 (hereafter, CF20) (October 2014), 5 stations from *Golfo de Penas* (47°S) to Baker Channel (48°S); (ii) CF23 (October 2017), 27 stations from *Golfo de Penas* (47°S) to *Estrecho Nelson* (51°S); and (iii) CF25 (September - October 2019), 6 stations from *Canal Concepción* (50°S) to *Golfo Almirante Montt* (52°S) (Figure 1, Suppl. Material). The megafauna database also draws from CF3, CF7, and CF15, which took place on board *RV* AGOR Vidal Gormaz (Rios et al., 2005, 2013) from *Golfo de Penas* (47°S) to Magellan Strait (53°S) (Figure 1). All samples were at depths between 24 and 1690 m, with the latter among the deepest ever for Chilean inner fjords. Bottom deployment time for each haul averaged 10–15 min. Information on trawl sampling stations is detailed in Supporting information Table S2). All specimens were preserved on board in 10% buffered formaldehyde. Megafauna were identified to the lowest possible taxonomic level using related literature (Rios et al., 2005, 2013; Häussermann and Försterra, 2009, Cari et al., 2020). For some taxa (e.g., Holothuroidea, Ascidiacea), identification was only possible at higher taxonomic levels.

2.4. Diversity patterns

Since macro- and megafauna samples were not necessarily of equal size, diversity patterns were determined by modified (ES_n) rarefaction method (Hulbert, 1971). Here, a species abundance data matrix was transformed according to $y=\sqrt{\sqrt{x}}$ under the ordination method for nonmetric multidimensional scaling (nMDS) analysis (Clarke and Gorley, 2006). Significant differences among stations were then determined using one-way permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001). nMDS analysis and rarefaction curves were carried out using Past 4.03 version statistical software (Hammer et al., 2001). Finally, a one-way ANCOVA distinguished effect from freshwater river inputs on the fauna isotopic composition among sites i.e., inner, middle and outer fjord sections. This analysis was proposed in order to eliminate the effects of the environmental conditions on the faunal stable isotopes signatures.

2.5 Carbon and nitrogen stable isotope analysis

Specimens were rinsed with distilled water for 24 h to evacuate gut contents (Levin and Currin, 2012). Muscle tissues were dissected in large individuals (e.g. holothurians, fishes), while the whole body was used in small individuals (e.g. amphipods, isopods, polychaetes). Specimens were stored whole at -20°C immediately after collection and prior laboratory analysis. Freeze-dried tissue samples were ground into a fine, homogeneous powder using an agate mortar and pestle, cleaned with methanol between grindings. Since no calcified tissues were present, no HCl treatment was needed. Lipids were not extracted prior ^{13}C stable isotope analyses: although ^{13}C tends to deplete in lipids, and suggested by some to introduce bias, its effect on nitrogen signature is still under debate (Mintenbeck et al., 2008). We rely instead on mathematical normalization, which considers the carbon-to-nitrogen (C/N) ratio of a sample to normalize $\delta^{13}C$ after analysis (Logan et al., 2008). This normalization is a lipid-correction equation for aquatic invertebrates: $\delta^{13}C_{lip\text{-}corr} = \delta^{13}C + 3.3338 - ((3.388 \times 3.314)/C/N)$.

Sediment OM samples were kept frozen (-20°C) prior to analyses of organic matter, carbon and nitrogen stable isotopes ratios. Stable isotope analysis, including carbon and nitrogen concentration measurements, was carried out at the Laboratory of Applied Stable Biogeochemical and Isotopes (LABASI, PUC, Chile) using an Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, Delta V Advantage IRMS) coupled with an Elemental Analyzer (Flash, EA 2000). Expressed in delta notation ($\delta^{13}C$ and $\delta^{15}N$), stable isotope ratios were measured, for carbon, as deviation from conventional standard Vienna Pee Dee Belemnite (VPDB); and for nitrogen, from atmospheric N_2 (‰).

3. Results

3.1. Diversity patterns

The Agassiz trawl study sites showed low numbers of species and individuals. Epibenthic fauna species ranged from 61 (CF15) to 122 species (CF23); and individuals, between 838 and 4055 on CF7 and CF25, respectively. In general, megafaunal communities were widely dominated by echinoderms, which contributed over 43% in terms of individuals. The most abundant faunal groups in the study areas were Ophiuroidea (23.19%), Asteroidea (20.29%), Polychaeta (8.95%), Bivalvia (8.95%) and Decapoda (8.85%) (Figure 2). Total species

richness (numbering 3 – 40 species) and abundance (4 – 2045 individuals) were higher in marine environments than in SPI glaciomarine systems (1 – 25 species; 1 – 1338 individuals) and hypoxic stations sampled during CF25 (4 – 15 species; 170 – 179 individuals) (Figure 3). Species compositions were dominantly Echinodermata (e.g., *Ophiuroglypha lymani*, *Ophiactis asperula*, *Ophiophragmus chilensis*, *Astrotoma agassizii*, *Cosmasterias lurida*, *Ctenodiscus procurator*, *Arbacia dufresnii* and *Pseudechinus magellanicus*), Mollusca (*Delectopecten vitreus*, *Cyclocardia compresa*, *Eunucula grayi*, *Fissidentalum majorinum*, *Limopsis marianensi* and *Lucinoma antarctica*) and Polychaeta (Onuphidae, Lumbrineridae, Spionidae, Polynoidae, Sigalionidae and Terebellidae; Supporting information Table S3).

There was no difference in rarefaction curves for epibenthic fauna richness (ES_{100}) between marine and glaciomarine environments (Figure 3). However, species number and diversity increased toward (ES_{100}=32) the Strait of Magellan areas (CF3–7) and decreased at hypoxic stations sampled during CF25 (ES_{100}=10). A similar pattern was identified in nMDS analysis, with significant differences for marine and glaciomarine groups (CF3–7, CF15, and CF25), mainly defined by the occurrence of echinoderms (i.e., Ophiuroidea and Asteroidea); but not during CF23, which registered an important contribution of Polychaeta and Bivalves (Figure 3; PERMANOVA test, $p<0.05$).

A total of 120 macroinfauna species were recorded in the MBF, mostly Polychaeta (58% of total abundance) with *Levinsenia antarctica* (14.51%), *Capitella* sp. (14.38%), *Aphelochaeta cf. marioni* (12.55%), *Aricidea antarctica* (8.88%), and *Prionospio (Minuspio) chilensis* (7.35%) as the most abundant. Rarefaction curves increased from inner (ES_{100}= 23) to outer (ES_{100}=30) fjords. nMDS showed higher dissimilarities among inner fjords, likely associated with higher ecological dominance of small-bodied Polychaetes (Figure 3; Supporting information Table S4; PERMANOVA test; $p<0.05$).

3.2. Stable isotopes ratios

Surface sediment C/N ratios and carbon and nitrogen stable isotopes exhibited well-defined longitudinal gradients in the MBF (Figures 4). The inner fjord (Martínez Channel) and proglacial Jorge Montt fjord were characterized by lower values of $\delta^{13}C$ (-23.99 to -29.36‰); and higher values in the outer fjords (*Golfo de Penas*, Martínez and Baker channels), from -20.89 to -22.37‰ (Kruskal-Wallis test; Hc=30.7; $p<0.05$). Nitrogen stable isotopes ($\delta^{15}N$)

showed lower values (-2.39 to 7.63‰) in stations at both inner and proglacial fjords (Kruskal-Wallis test, Hc=21.9; Dunn's post hoc test, $p<0.05$). C/N ratios varied from 1.71 to 4.03 in inner and proglacial fjords, respectively; and, although higher at outer fjords, not significantly (C/N=8.35-8.71; Kruskal-Wallis test, Hc=22.6; $p<0.05$).

Spatial isotopic characterization of megafauna and macrofauna, excluding feeding mode and taxonomic composition, showed depleted values (-15.49 to -26.3‰) of $\delta^{13}C_{lipid\text{-}corr}$ in the inner fjord (Martinez Channel); and, in middle and outer fjords (*Golfo de Penas*, Martinez and Baker channels), enriched values of δ^{13} $C_{lipid\text{-}corr}$, ranging from -7.01 to -22.7‰ (Kruskal-Wallis test, Hc=56.9; Dunn's post hoc test, $p<0.05$). Faunal stable nitrogen isotope composition ($\delta^{15}N$) showed lower values (1.68 to 14.8‰) at inner fjord stations (Kruskal-Wallis test, Hc=40.9; $p<0.05$). In contrast, $\delta^{15}N$ in both middle and outer fjords varied from 4.88 to 19.93‰ (Figure 5; Supporting information Table S3). C/N ratios varied from 2.78 to 15.3 with no statistically significant differences (Kruskal-Wallis test, Hc=3.86; $p>0.05$). Distribution of isotopic signatures in fauna was consistent with isotopic compositions among sampling sites (Figure 5), indeed, with significant spatial variation (ANCOVA test, $F_{2\text{-}187}$ = 12.3; $p<0.001$; Supporting information Table 5).

Isotopic characterization of megafauna, with taxonomic composition, is shown in Figure 10. It showed depleted values of $\delta^{13}C_{lipid\text{-}corr}$ (-11.4 to -26.3‰) for Gastropoda (*Chilina chilensis*) and Polychaeta (Lumbrineridae unidentified), while Bivalvia exhibited enriched values of δ^{13} $C_{lipid\text{-}corr}$, from -8.3 to -18.8‰ (Kruskal-Wallis test, Hc=45.1; $p<0.05$). Megafaunal stable nitrogen isotope ($\delta^{15}N$) compositions showed lower values, varying from 6.39 to 16.9‰ for Echinodermata and Nemertinea; and for Bivalvia, in contrast, from 8.27 to 11.2‰ (Figure 6; Supporting information Table S3; Kruskal-Wallis test, Hc=57.1; $p<0.05$). C/N ratios were lower for Gastropoda (3.4 to 5.3) and higher for Bivalvia (3.5 – 14.6) (Kruskal-Wallis test, Hc=84.1; $p<0.05$). The distribution of these signatures was consistent with isotopic compositions among faunal groups (Figure 6).

Isotopic feeding modes composition showed depleted values (-7.4 to -26.3‰) of $\delta^{13}C_{lipid\text{-}corr}$ in deposit feeders, and enriched values in herbivores (-12.8 to -21.0‰) (Kruskal-Wallis test, Hc=9.1; $p<0.05$). Stable isotope nitrogen composition ($\delta^{15}N$) was lower in herbivores (8.9 to 10.2‰), and slightly higher in carnivores (6.4 to 16.9‰) (Figure 6; Kruskal-Wallis test, Hc=16.2; $p<0.05$). C/N ratios were higher in deposit feeders (2.8 to 15.3)

and lower (3.5–4.2) in herbivores (Kruskal-Wallis test, Hc=41.5; $p<0.05$). The analysis mixing polygon shows deposit feeder isotopic signatures within the range of food sources (Figure 6).

4. Discussion

4.1. Sedimentary environments

Although freshwater inputs from glaciomarine and glaciofluvial systems are key drivers behind steep environmental gradients in sedimentation and salinity in Patagonian fjords, few studies on benthic communities discuss this issue (e.g., Silva et al., 2011; Quiroga et al., 2016; Bianchi et al., 2020). Furthermore, accumulation of organic carbon (C_{org}) and burial rate in sediments are key factors on the contribution of OM to the seafloor, being one the most important food supply for benthic deposit-feeder organisms. The SOM isotopic composition in our study area showed steep environmental gradients, demonstrative of the supply dynamics between riverine organic matter and horizontal and vertical transport of particles in the water column, driven by tidal mixing (Rebolledo et al., 2019). In the MBFs, SOM $\delta^{13}C$ was depleted in inner-glacier fjords (mean -26.5‰) and enriched in outer fjords (mean -23.3‰), similar to other Patagonian fjords (mean -26.7‰; Zapata-Hernández et al., 2016), Arctic fjords (mean -25.2‰ to -27.4‰; Włodarska-Kowalczuck, 2019a), and Canadian fjords (-22.1 to -22.6‰; Muzuka and Hillaire-Marcel, 1999). These surface sediment values indicate significantly allochthonous OM (Silva et al., 2011; Rebolledo et al., 2019).

In outer fjords, however, sedimented POM $\delta^{13}C$ (-20.9 to -22.0‰) were within expected values for primary production OM. Indeed, higher chlorophyll-a content was registered in outer fjord surface sediments (1.33 to 7.48 µg g^{-1}), reflecting vertical export of phytoplankton biomass to sediments (Quiroga et al., 2016). The $\delta^{15}N$ in surface sediments showed similar trends, with higher values in the outer fjord (mean 8.12‰) and lower values in glacier fjords (mean 2.7‰), likely due to low inputs of labile OM (Włodarska-Kowalczuck, 2019a). The C/N ratio, an indicator of OM degradation, showed higher values in outer fjords (mean 8.41), suggesting recent deposits of material (e.g., phytoplankton aggregates). The above trends are supported by previous studies on vertical flux of organic carbon (C_{org}) in the MBF, varying between 13-112 mg C m^2d^{-1} in the inner-middle fjords,

and 559 mg C m^2d^{-1} in the outer fjords (Quiroga et al., 2016). While these values are similar in both glaciomarine (3-22.8 mg C m^2d^{-1}) and non-glacier influenced environments in Patagonian fjords (168-725 mg C m^2d^{-1}; Quiroga et al., 2016), they are lower than in high latitude Arctic fjords and the Barents Sea (770-1530 mg C m^2d^{-1}), due probably to the higher influence of sediment-loaded glacial runoff there (Silva et al., 2011; Włodarska-Kowalczuk et al., 2019a).

Sediment stability – one of the most important factors in OM distribution and cycling (Quiroga et al., 2016) – can be disturbed by iceberg scouring, glacier plume sedimentation, and terrestrial debris subsidies (i.e., combinations of vascular woody plants and freshwater phytoplankton) (Quiroga et al., 2016; Rebolledo et al., 2019). While these physical mechanisms may explain the OM distribution described above, we should consider the C_{org} content, origin, and burial rates to determine benthic mineralization pathways (Faust and Knies, 2019; Włodarska-Kowalczuk et al., 2019a). Measurements in the MBF for C_{org} (mean 17.1 mg g^{-1}; Sepulveda et al., 2011) and terrestrial C_{org} (39.4%) content were similar to those registered in Arctic fjords (19 mg g^{-1}; 32.5%; Włodarska-Kowalczuk et al., 2019a). However, burial rates of marine and terrestrial organic carbon are lower in Patagonian fjords (marine C_{org} burial rate= 10.3 gC m^2y^{-1}; terrestrial C_{org} burial rate= 5.9 gC m^2y^{-1}) than in Arctic fjords (marine C_{org} burial rate= 17 gC m^2y^{-1}; terrestrial C_{org} burial rate= 9.5 gC m^2y^{-1}; Sepúlveda et al., 2011; Włodarska-Kowalczuk et al., 2019a; Supporting information Table S6). Burial rates are mainly influenced by oceanographic conditions – i.e., whether the interaction between oceanic and freshwater waters produces strong vertical and horizontal gradients in salinity, density, organic and inorganic nutrient ratios.

In the MBF, lateral advection and sedimentation of terrestrial OM contributed with a significant fraction of total OM, varying between 77-93% in the inner fjord and 1-15% in the outer fjord (Quiroga et al., 2016). tOM estimates - based on Bayesian adjustments of Bianchi – indicate that about 32 and 50% contributed to the macrofauna diet. This shows that tOM plays an important role in the benthic food web. As associated with sediment-load environmental disturbances, such terrestrial OM input to the inner fjords is likely the cause of higher trophic redundancy and lower alpha diversity in the benthic communities, with fewer trophic levels in comparison to no-glacier environments (Rebolledo et al., 2019; Cari et al., 2020).

4.2. Soft-bottom benthic communities

There are several hypotheses for the distinct regional benthic biodiversity patterns in Chilean waters. Moreno et al. (2006) hypothesized that benthic polychaetes endemism is a recolonization from paleo refugium – where species escape severe extinction events (i.e., glaciations). The large-scale polychaetes distribution is also hypothesized from ecological drivers, such as larval meroplanktonic distributions (dispersalist hypothesis; Montiel et al., 2005). Habitat heterogeneity and oceanographic conditions (i.e., temperature) have also been proposed to explain local and large-scale benthic diversity patterns, particularly in shallow-water species, due to habitat types, species refuges, and speciation (Häussermann and Försterra, 2009, Stein et al., 2014). We argue that environmental stress produces changes in both the environmental condition and nutrients input, influencing the benthic diversity patterns based on the quality of OM. For example, both invasive organism success – high performance, growth, and production – and species richness have been associated with ability to change nutrient requirements. Such changes are evident in community stoichiometry, altering C/P and N/P ratios (Sterner and Elser, 2002; Van de Waal et al., 2018). The environmental stress is especially useful in Patagonian fjord benthic communities, where quality and quantity of food resources appear through complex interactions among environmental (e.g., freshwater input and hydrography) and ecological factors (e.g., primary production, terrestrial OM input) at different spatial scales (Quiroga et al., 2016; Cari et al., 2020; Friedlander et al., 2021). The resulting species composition and relative abundance in mega- and macrobenthic communities appear to be dominated by echinoderms (asteroids and ophiuroids) and polychaetes, respectively. In the MBF, species richness was, on average, 220 species, with marked changes between inner and outer fjords (Quiroga et al., 2016). Our nMDS analysis supported the correlation between the well-defined changes in community structures and specific environmental conditions. Glaciomarine environments showed lower species richness as a function of high levels of suspended sediment. Although the local effects of small iceberg scouring – in glacier fronts or in shallow water – on benthic communities are still little understood, ecological information from Antarctic fjords suggests that the environmental conditions produced by local glacial melt-related retreat may influence lower trophic levels, playing an important role in organic carbon cycling (Alurralde et al., 2020; Barnes et al., 2018, 2020). Our results are consistent with species diversity patterns from the

inner-to-outer Arctic fjord as responses to environmental gradient (Włodarska-Kowalczuk et al., 2012). Benthic macrofauna diversity and functional traits appear to be similar, suggesting that environmental stress drives macrofaunal adaptations to sediment material load and limited OM inputs (Włodarska-Kowalczuk et al., 2012; Quiroga et al., 2016).

Furthermore, studies indicate that individual metabolism and stable isotope signatures are correlated with environmental nutrient availability and POC/PON reservoirs (Hunter et al., 2012). Benthic fauna in our study area – dominated by deposit-feeders, suspension feeders, and carnivores – had $\delta^{13}C$ and $\delta^{15}N$ values that were highly consistent with those of sediment organic matter along the fjords, confirming the importance of terrestrial OM in the food web (Figure 7; Supporting information Table S7). Additionally, water column differences in nutrient concentrations (N/P) and POC and PON reservoirs in the Martinez Channel were clearly related to freshwater glaciofluvial discharges and tOM. By modifying the biogeochemical properties of water columns and sediments, these differences have shaped spatial and temporal benthic patterns (Quiroga et al., 2016; Cari et al., 2020).

Although the above supports the close relationship between OM and nutrient availability with faunal isotopic composition, we found a decoupling in C/N ratios between fauna and sediment (Figure 7; Supporting information Table S7). Briefly, OM availability for biological utilization (lability) is strongly correlated with relative organic nitrogen content, with C/N ratios in somatic tissues of both marine and freshwater benthic fauna ranging from 1.5 and 9 (Hunter et al., 2012). Surface sediment OM in our study area had C/N ratios over 6.6, similar to those of other fjords in the region. This has been suggested as a result of low organic nitrogen content in edaphic material transferred to sediment OM (Silva et al., 2011, Rebolledo et al., 2019). Indeed, although studies addressing C and N coupling are scarce, they have argued that biogeochemical decoupling between food supply and fauna-assimilated OM is related to metabolism. As a result, decoupled C/N ratios between sediment OM and benthic fauna explain that nitrogen is a limiting nutrient in marine sediments, hence benthic fauna preferentially process C_{org}. In this sense, Bracken (2017) studied stoichiometric mismatch between consumers (bivalves) and resources (Chl-a and POC) measured by mussel growth rates under different POC and PON availability and found that elemental ratios in consumers were more different than their resources, providing further evidence that stoichiometry mismatch depends mainly on the OM available.

Then, biogeochemical decoupling may be identified using elemental ratios such as C/N or $\delta^{13}C/\delta^{15}N$. In the MBF, organismal C/N ratios varied from 4.28 (±0.29) in the inner fjord, to 4.60 (±0.18) in the outer fjord. These values are within reported C/N ratios for the Patagonia with mean values 4.26 ±0.19 for Reloncavi fjord, 4.16 ±0.19 for Interior Sea of Chiloé, and 4.52 (±0.08) for Puyuhuapi fjord (Figure 8). While these values indicate that OM originates from primary production and terrestrial plants and debris, the MBF C/N ratio – with its low level of nitrogen – appears to be associated with terrestrial OM and glacier activity (Rebolledo et al., 2019). The higher C/N ratio in the Puyuhuapi fjord seems to be related with allochthonous nitrogen inputs; indeed, salmon farming activities and nitrogen inputs associated with aquaculture likely play an important role in OM cycling (Wang et al., 2012).

As mentioned above, the biological processes of production and metabolism have been used to explain patterns across taxa or communities. This is as true for ecosystems as for individuals that metabolic ecology theory is an important and powerful approach to understanding the ecology and evolution of marine ecosystems (Brown et al., 2004). Therefore, food limitations drive geographic variations across life histories, size, abundance, and diversity (O'Connor and Bruno, 2012). In our study area, glaciomarine and glaciofluvial environmental stress o negatively affected nutrient reservoirs and OM availability, and hence changed the benthic diversity and trophic structure throughout the fjords (Alurralde et al., 2020; Barnes et al., 2020; Cari et al., 2020). These conditions have shaped a benthic community dominated by small-bodied polychaetes (*L. antarctica, A. marioni,* and *Capitella capitata*); opportunistic species, like decapods (i.e., *Libidoclaea granaria, Peltarion spinulosum*); and echinoderms (*Tripylaster philippii*). In addition, we demonstrated the importance of integrating community diversity patterns and stoichiometric composition across multiple trophic levels to identify ecosystem-wide responses. These approaches help us to understand complex ecological and biogeochemical interactions within ecosystems and are necessary in assessing the ecological consequences of abiotic environmental changes on marine benthic communities.

Soft-bottom epibenthic fauna and macroinfauna in Chilean fjords are intimately connected to oceanographic conditions and suspended sediments, particularly in the inner fjords. Since the Chilean fjord region has proven to be one of the most sensitive to climate

change (Iriarte et al., 2018), consistent records of its benthic marine biodiversity patterns, isotopic compositions, and stoichiometric imbalances will be useful ecological indicators at global level. They will additionally act as bellwether for possible environmental impacts in the coming decades from expected shifts in rainfall regimes and air temperature in the region (Garreaud et al., 2013). Such changes in freshwater inputs and nutrients will certainly negatively affect primary productivity, particularly in glacial-influenced environments, with apparent consequences to the biodiversity and functioning of Patagonian benthic ecosystems.

4.3. Conclusions

We have shown how the complex interaction between hydrographic and ecological factors at different spatial scales influence the quality and quantity of food resources for benthic communities in Patagonian fjords. Soft-bottom benthic (i.e., mega- and macroinfauna) communities exhibited a species richness gradient across the inner and outer fjords, mainly dominated by echinoderms (asteroids and echinoids) and polychaetes. This faunal structure correlates to the distribution of tOM in sediments, which gradually decrease towards the outer fjord. There is furthermore a C/N ratio decoupling in benthic fauna, suggesting preferential organic carbon use under limited nitrogen in fjord sediments.

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1. Figures

Figure 1

(A) Location of SPI sampling stations: Agassiz trawl (AGT) from CIMAR fjord program, CF25 (red circles), CF23 (yellow circles), CF15 (orange circles), CF7 (green circles), and CF3 (blue circles). (B) Location of Martínez-Baker fjords complex (MBF) sampling stations. The North (NPI), South (SPI), and Cordillera Darwin (CDI) Icefields, and other smaller mountain glaciers, are shown in box for comparison.

Figure 2

Megafauna relative abundance (%) for main taxonomic groups. Total individuals and number of species per cruise.

Figure 3

Left panel: Rarefaction (ES) curves for SPI megafauna (CF3-7, CF15, CF23, CF25 and MBFs). Right panel: Non-Metric multidimensional scaling (nMDS) ordination for SPI megafauna (CF3-7, CF15, CF23, CF25 and MBFs). Glacial (red circles) and marine environments (blue circles). PERMANOVA Test ($p<0.05$).

Figure 4

Stable isotopes of (A) Carbon ($\delta^{13}C$) and (B) nitrogen ($\delta^{15}N$); (C) C/N ratios versus longitude (°W); and (D) mixing polygon of carbon and nitrogen stable isotope ratios. SOM sampled from Martínez-Baker fjords complex: Glacial fjord (gray circles), inner fjord (blue circles), middle fjord (black circles), and outer fjord (green circles).

Figure 5

Comparison of the measured $\delta^{13}C_{lipid\text{-}corr}$; $\delta^{15}N$; C/N values for megafauna grouped by location from the Martínez-Baker fjords complex: Inner fjord (1), middle fjord (2) and outer fjord (3).

Figure 6

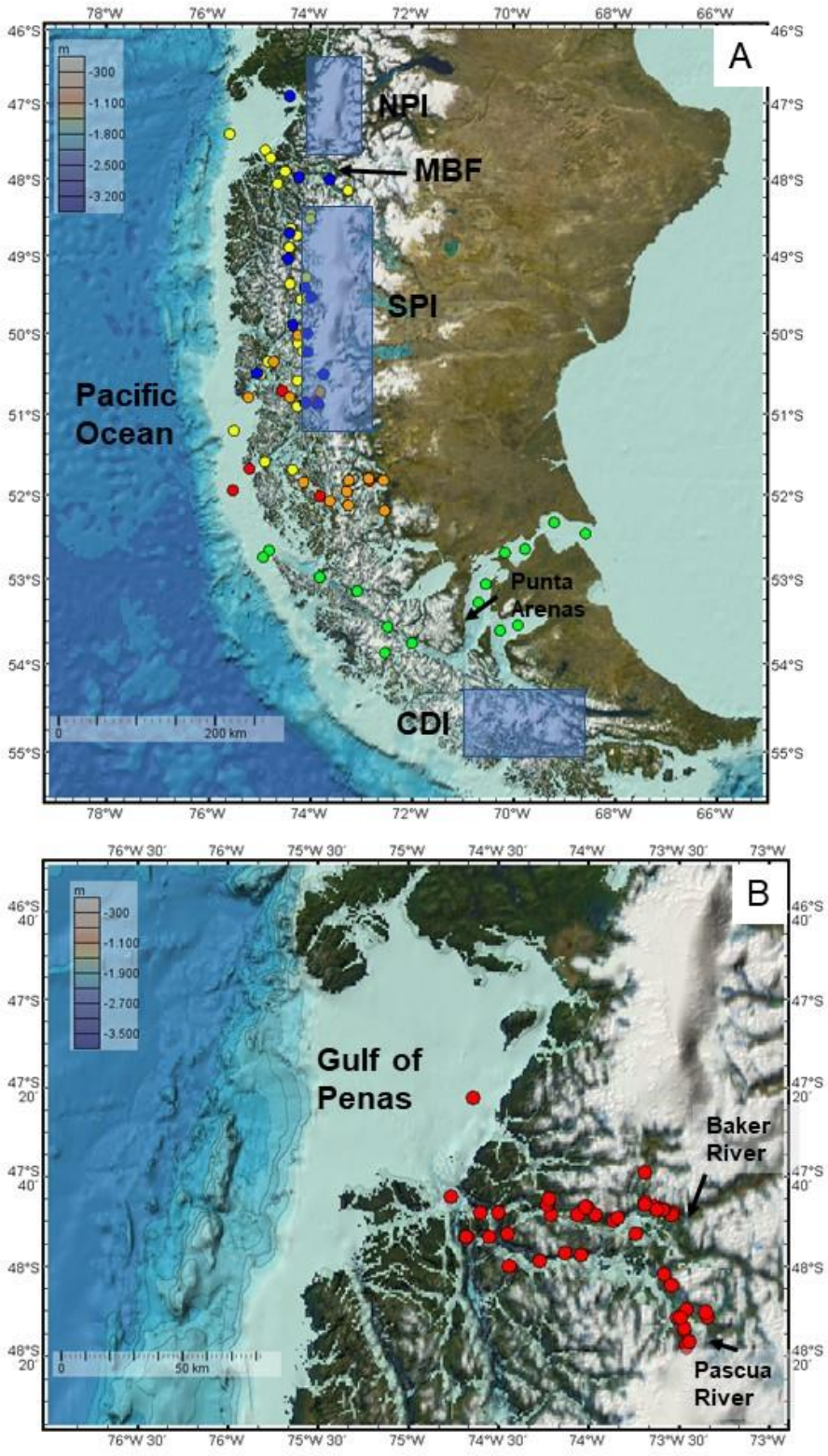
Upper panel: Comparison of the measured $\delta^{13}C_{lipid\text{-}corr}$; $\delta^{15}N$; C/N values for megafauna sampled from the Martinez-Baker fjords complex, by taxonomic groups (1, Bivalvia; 2, Decapoda; 3, Echinodermata; 4, Gastropoda; 5, Hydrozoa; 6, Nemertinea; 7, Peracarida; 8, Pisces; 9, Polychaeta; 10, Priapulida and Sipuncula). Lower panel: Comparison of the measured $\delta^{13}C_{lipid\text{-}corr;}$ $\delta^{15}N$; C/N values in megafauna sampled along the Martinez-Baker fjords complex, by feeding modes (1, Carnivores; 2, Deposit feeders; 3, Herbivores; and 4, Suspension feeders)

Figure 7

Diagram showing the (1) distribution of particulate organic carbon and nutrient N/P ratios in the water column; (2) distribution of isotopic composition and C/N ratio in SOM; and (3) biogeochemical and biological responses of epifauna and macroinfauna along the fjord. (a) *Tripylaster philippii*, (b) *Capitella capitata*, (c) *Peltarion spinulosum*, (d) *Levinsenia antarctica*.

Figure 8

C/N ratio of benthic organisms measured in Reloncavi fjord, inner Sea of Chiloé, Puyuhuapi fjord, and Martínez-Baker fjord systems (Database from: Zapata-Hernández et al., 2016; Cari et al., 2020 and unpublished data).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SPI
Pacific Ocean
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m
-300
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-1.800
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200 km
78°W
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74°W
72°W
70°W
68°W
66°W
46°S
47°S
48°S
49°S
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51°S
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54°S
55°S
B
Gulf of Penas
Baker River
Pascua River
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-1.100
-1.900
-2.700
-3.500
0
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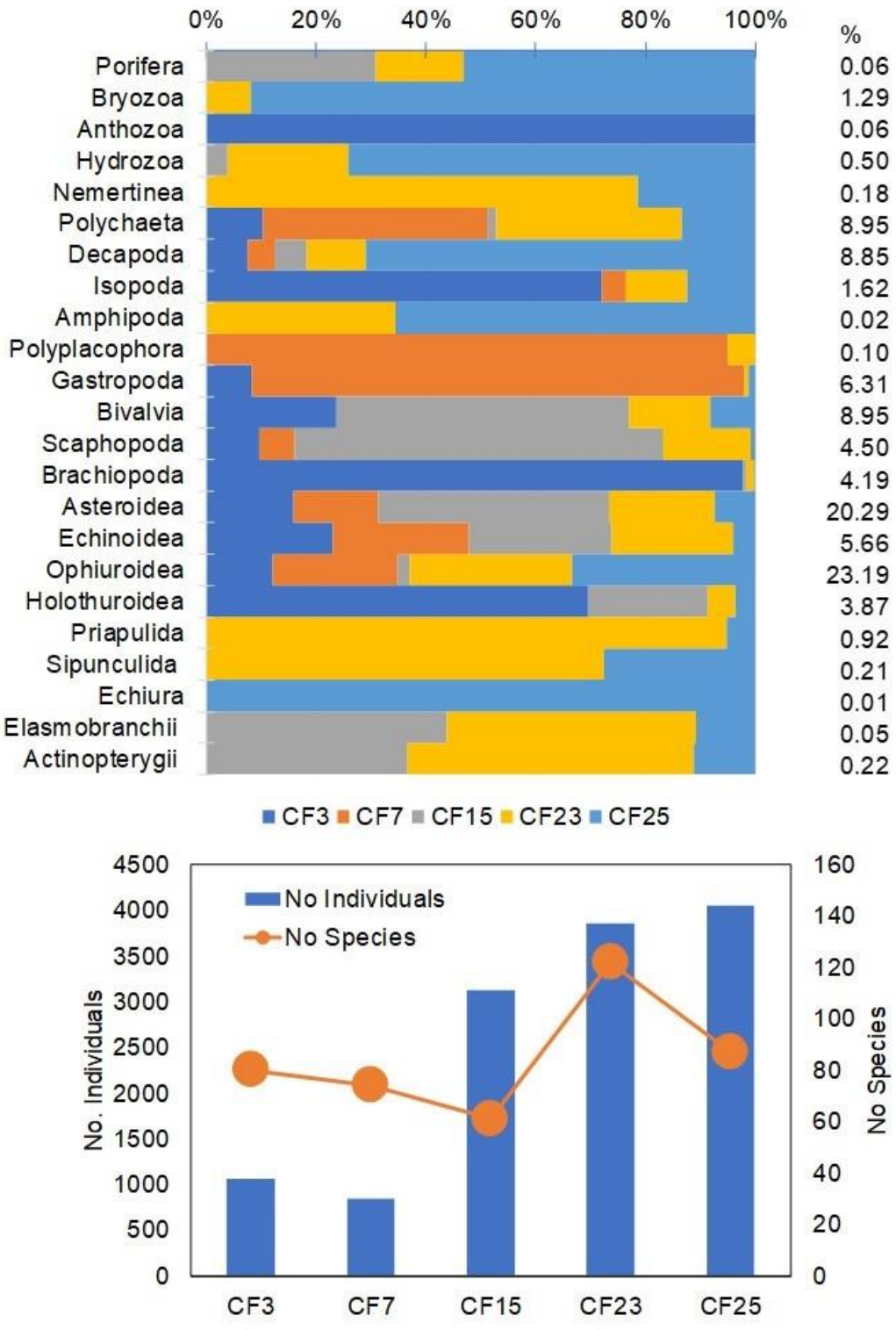
Fig. 1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Porifera 0.06
Bryozoa 1.29
Anthozoa 0.06
Hydrozoa 0.50
Nemertinea 0.18
Polychaeta 8.95
Decapoda 8.85
Isopoda 1.62
Amphipoda 0.02
Polyplacophora 0.10
Gastropoda 6.31
Bivalvia 8.95
Scaphopoda 4.50
Brachiopoda 4.19
Asteroidea 20.29
Echinoidea 5.66
Ophiuroidea 23.19
Holothuroidea 3.87
Priapulida 0.92
Sipunculida 0.21
Echiura 0.01
Elasmobranchii 0.05
Actinopterygii 0.22
CF3 CF7 CF15 CF23 CF25
No Individuals
No Species
No. Individuals
No Species
4500
4000
3500
3000
2500
2000
1500
1000
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CF3
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CF23
CF25

Fig. 2

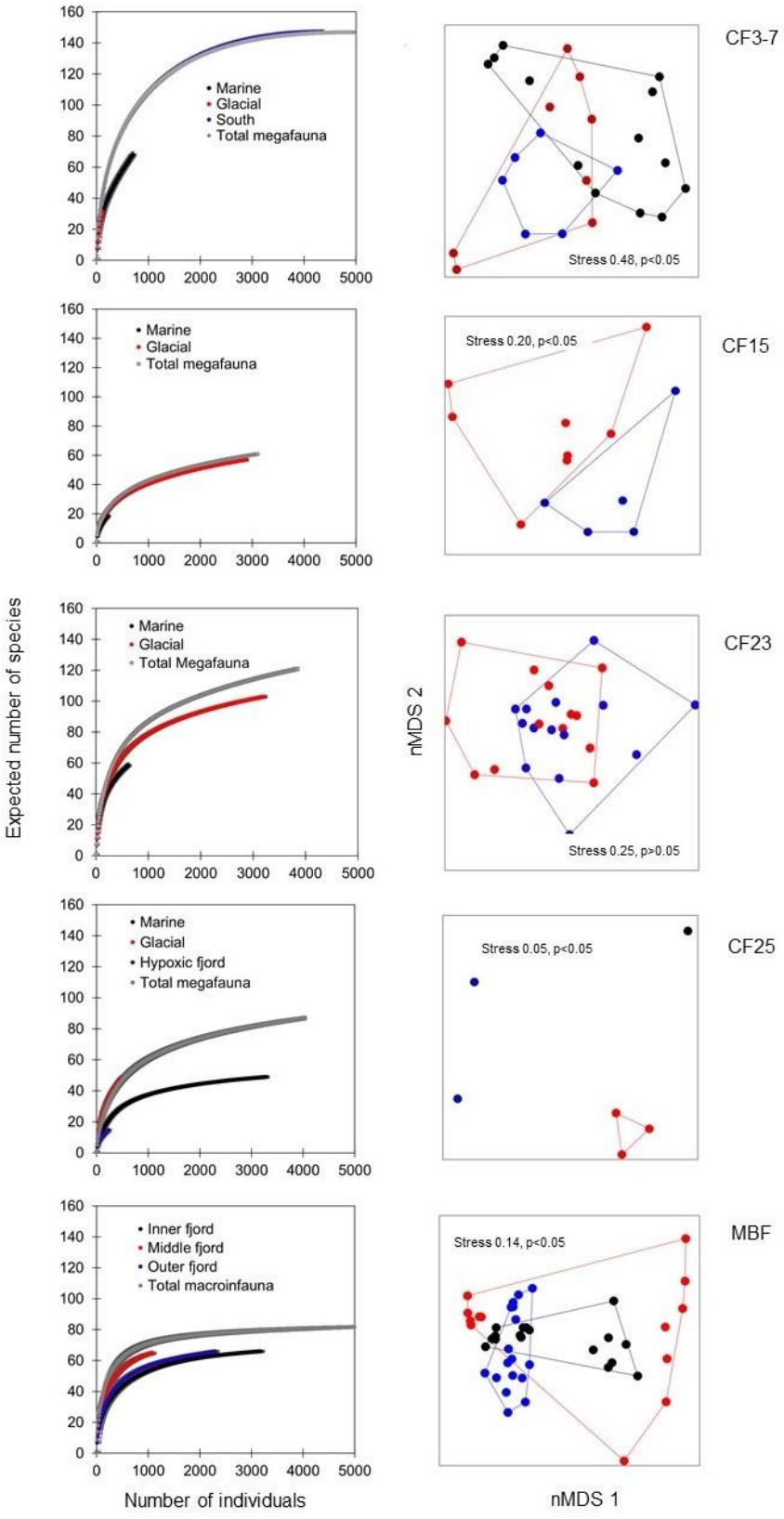

Expected number of species
Number of individuals
nMDS 2
nMDS 1
• Marine
• Glacial
• South
• Total megafauna
CF3-7
Stress 0.48, p<0.05
• Marine
• Glacial
• Total megafauna
Stress 0.20, p<0.05
CF15
• Marine
• Glacial
• Total Megafauna
CF23
Stress 0.25, p>0.05
• Marine
• Glacial
• Hypoxic fjord
• Total megafauna
Stress 0.05, p<0.05
CF25
• Inner fjord
• Middle fjord
• Outer fjord
• Total macroinfauna
Stress 0.14, p<0.05
MBF

Fig. 3

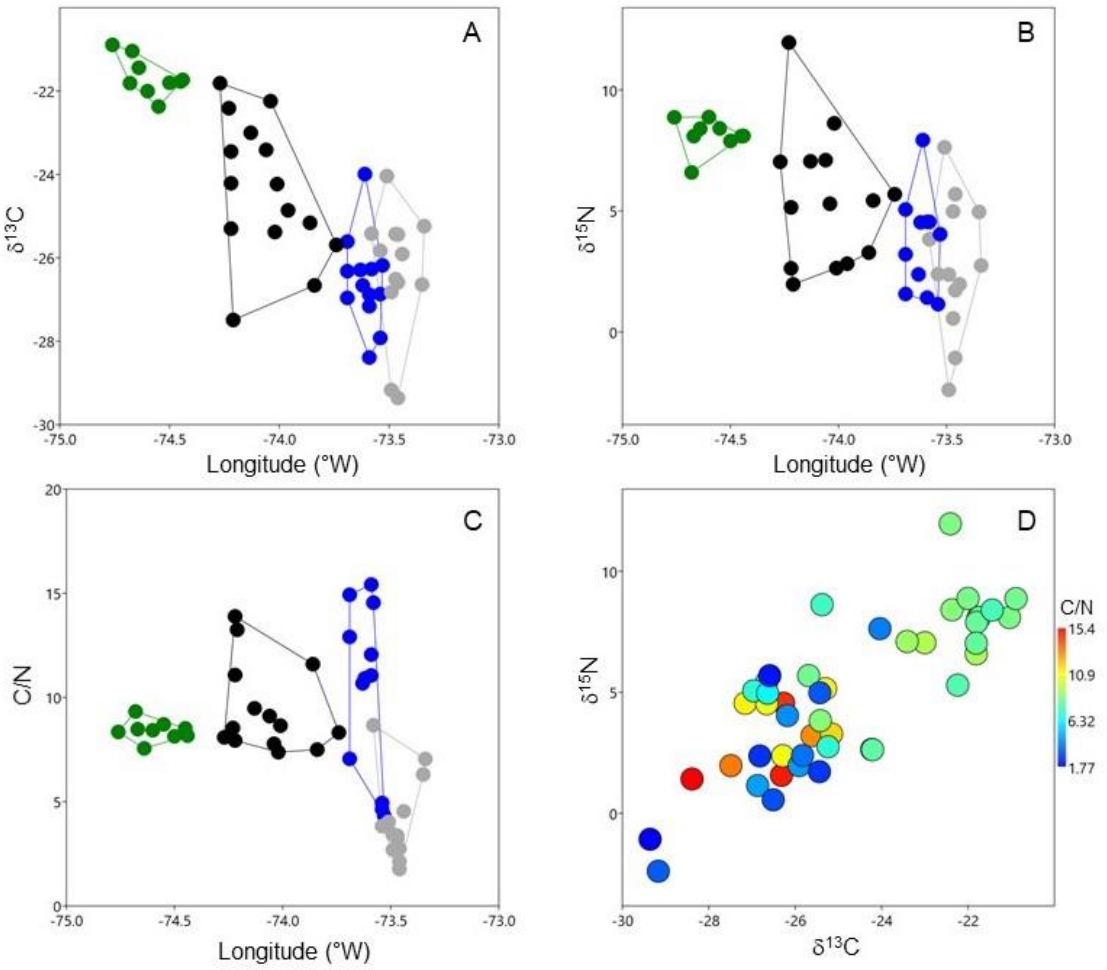
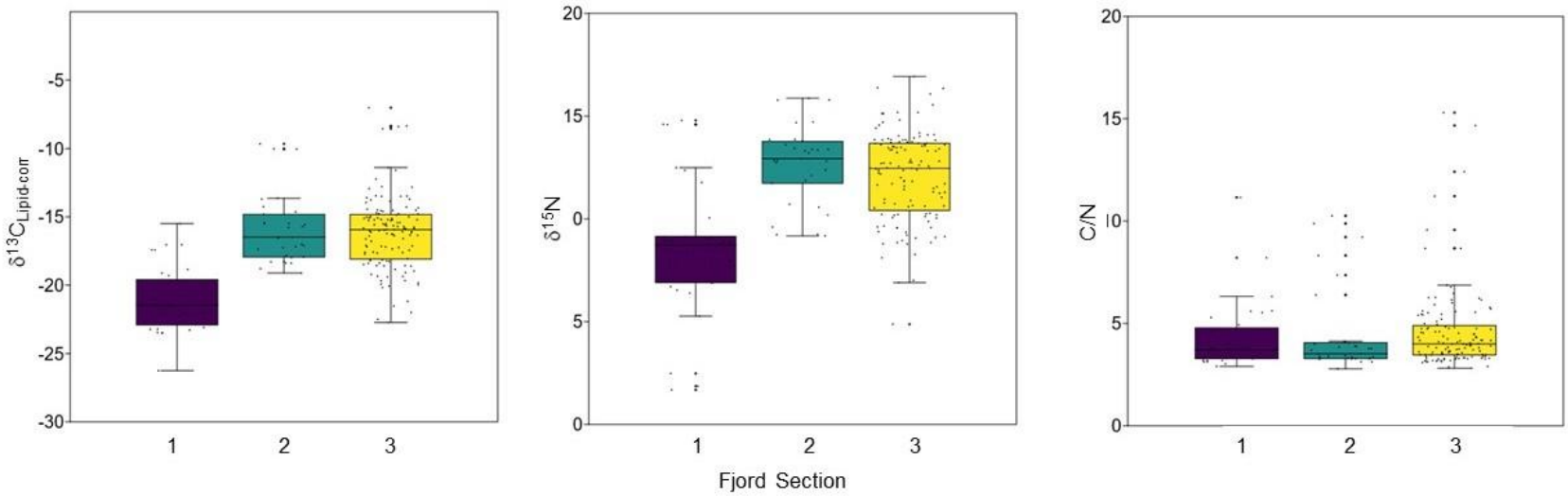

A
B
C
D
δ13C
δ15N
C/N
Longitude (°W)
-75.0
-74.5
-74.0
-73.5
-73.0
-22
-24
-26
-28
-30
10
5
0
20
15
10
5
0
15.4
10.9
6.32
1.77

Fig. 4


δ13CLipid-corr
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-10
-15
-20
-25
-30
1
2
3
δ15N
20
15
0
5
0
1
2
3
Fjord Section
C/N
20
15
10
5
0
1
2
3

Fig. 5

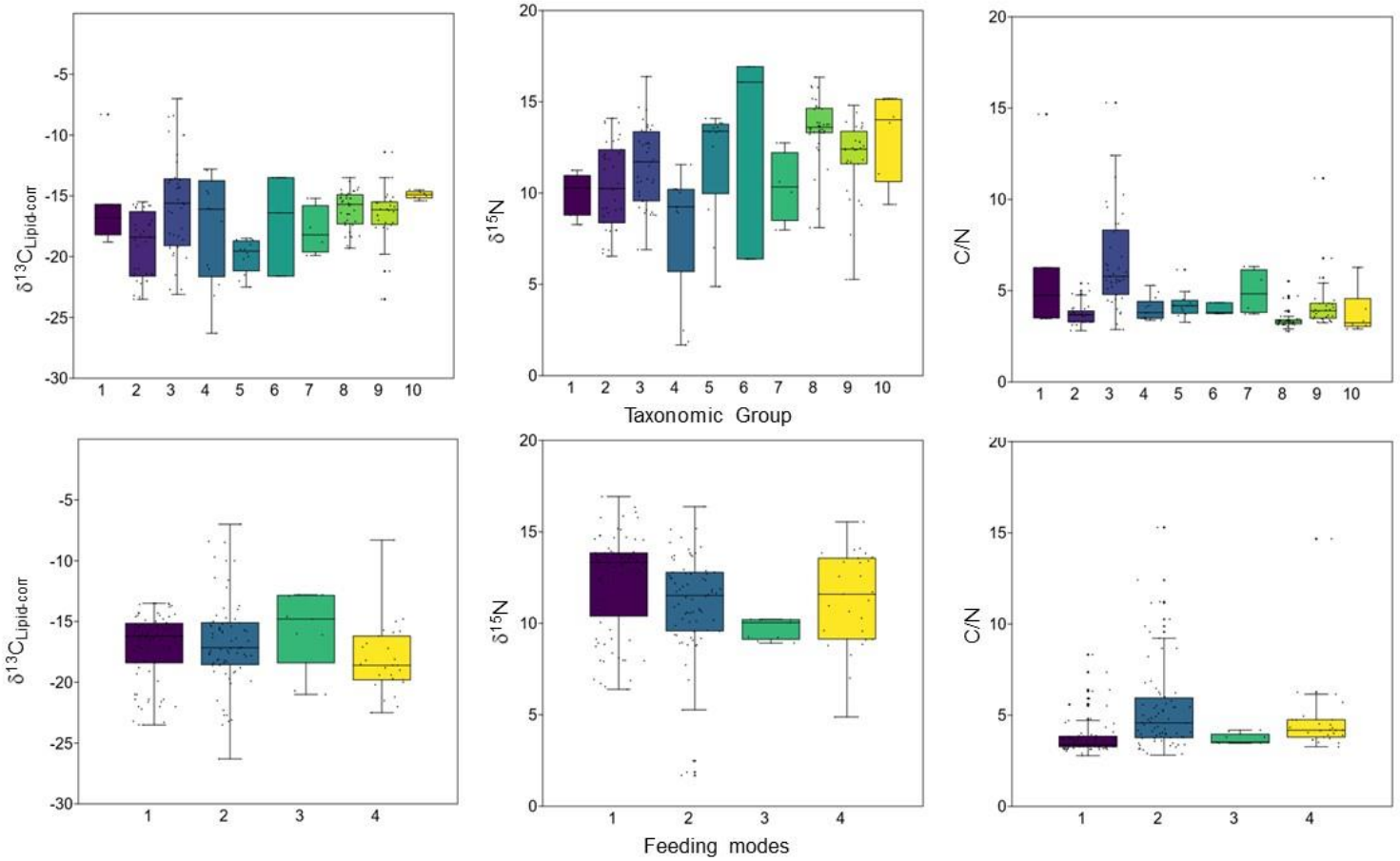

Fig. 6

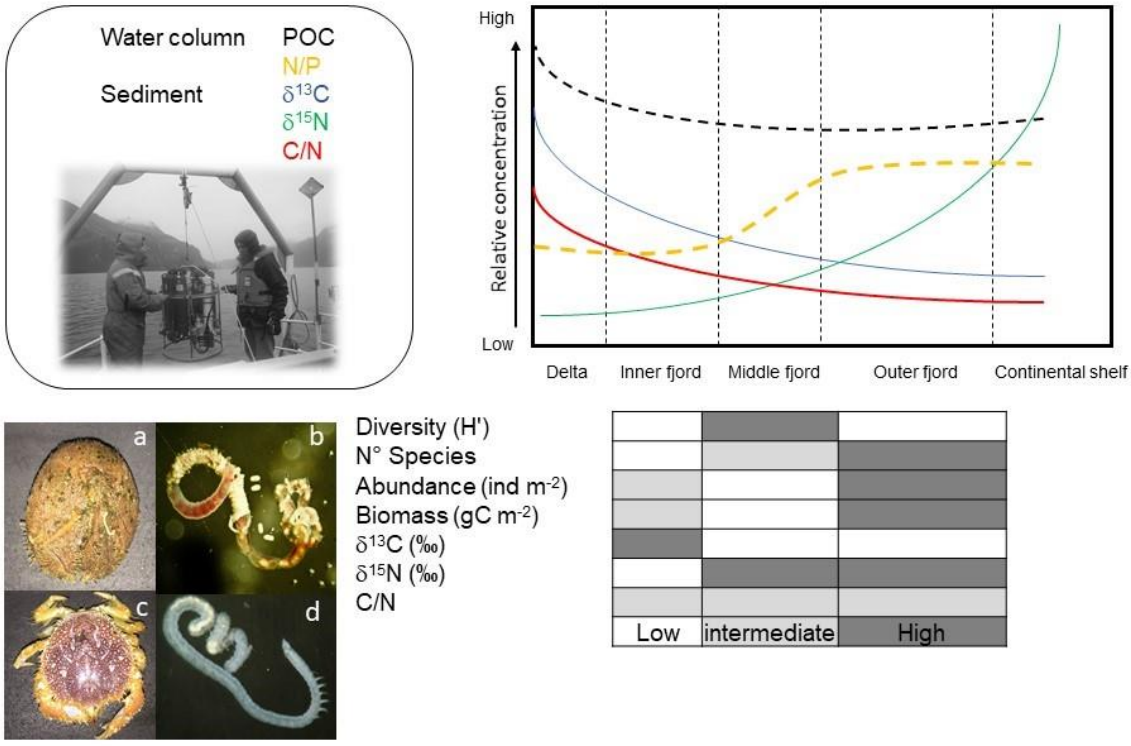
Water column
POC
N/P
Sediment
δ13C
δ15N
C/N
High
Relative concentration
Low
Delta
Inner fjord
Middle fjord
Outer fjord
Continental shelf
a
b
c
d
Diversity (H')
N° Species
Abundance (ind m-2)
Biomass (gC m-2)
δ13C (‰)
δ15N (‰)
C/N
Low
intermediate
High

Fig. 7

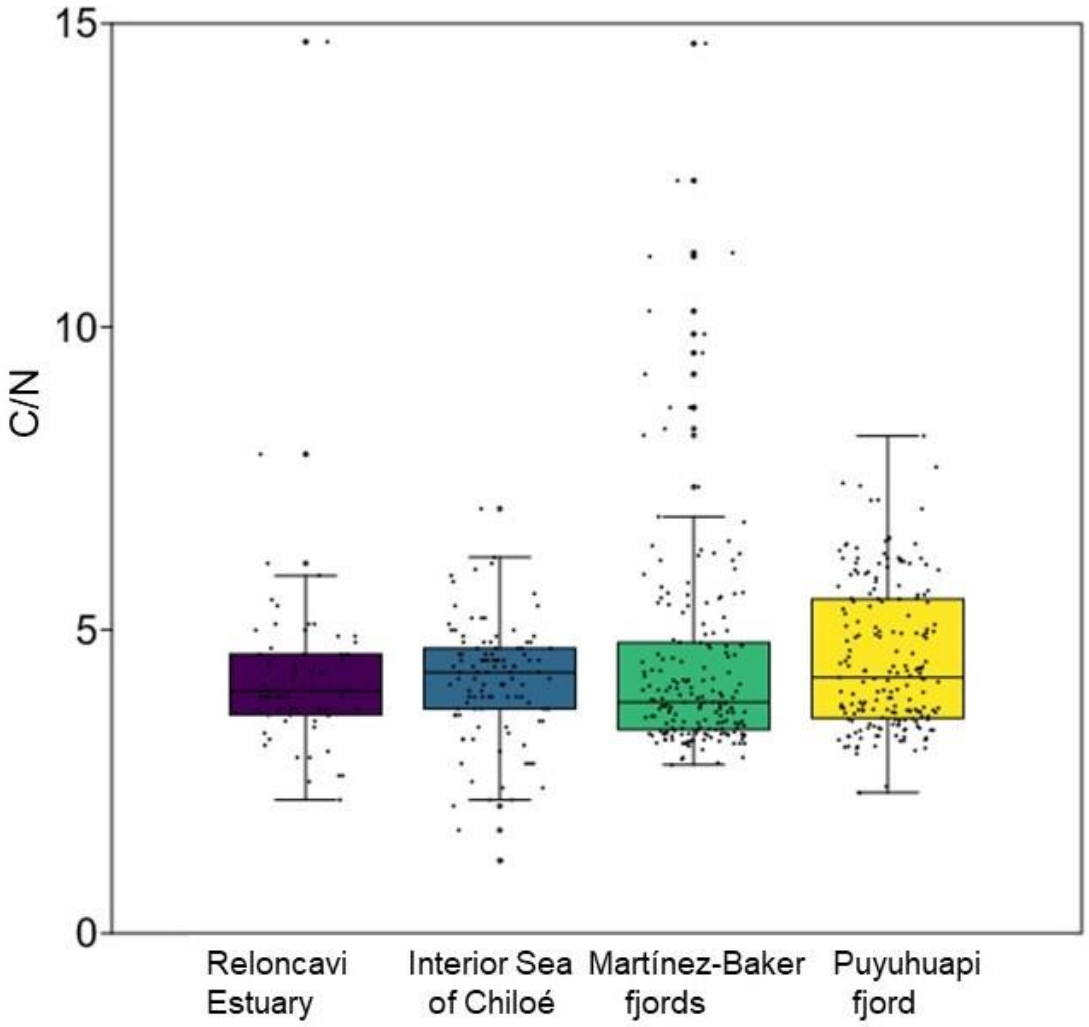
15
10
5
0
C/N
Reloncavi Estuary
Interior Sea of Chiloé
Martínez-Baker fjords
Puyuhuapi fjord

Fig. 8

7. Appendix A. Supplementary material

Supporting information Table S1 (MBFs sample stations, carbon and nitrogen stable isotopes, and sediment organic matter C/N ratios), Supporting information Table S2 (Agassiz trawl information, species number and relative abundance in the Southern Patagonian Icefield), Supporting information Table S3 (Pooled data for megafauna species composition and stable isotope values), Supporting information Table S4 (Pooled data for species composition, mean density, standard deviation (SD), and percent of total macroinfauna for the whole study period, Supporting information Table S5 (One-way ANCOVA for differences in fauna isotopic composition among MBFs sites), Supporting information Table S6 (Mean ±SD values of organic carbon (C_{org}), contribution of terrestrial C_{org} (%), marine and terrestrial C_{org} burial rates), and Supporting information Table S7 (Summary of biogeochemical and biological database for water column, sediment and fauna in the MBFs).

Table S1.

Cruise	Date	Location	Station	Environment	Latitude (°S)	Longitude (°W)	Depth (m)	C/N (molar)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
C14F	11-5-2008	Tortel	E12t	Middle	-47.81	-74.21	89	13.25	-27.49	1.97
C14F	11-4-2208	E. Steffen	E15	Inner	-47.65	-73.69	149	14.93	-26.32	1.57
C14F	11-4-2008	E. Steffen	E13	Inner	-47.78	-73.63	176	10.68	-26.29	2.38
C14F	11-4-2008	E. Steffen	E14	Inner	-47.76	-73.69	270	12.91	-25.61	3.21
C14F	11-3-2008	Canal Baker	E9	Glacier-fjord	-48.03	-73.58	380	8.67	-25.42	3.83
C14F	11-5-2008	Canal Martínez	E98a	Middle	-47.83	-73.86	286	11.60	-25.16	3.28
C14F	11-2-2008	Canal Baker	E7	Middle	-47.96	-74.04	700	7.78	-22.24	5.30
C14F	11-2-2008	Canal Baker	E6a	Middle	-47.98	-74.27	700	8.09	-21.81	7.03
C14F	11-6-2008	Canal Martínez	E4p	Outer	-47.80	-74.50	550	8.14	-21.80	7.89
C14F	11-1-2008	Canal Baker	E5	Outer	-48.00	-74.44	674	8.17	-21.73	8.10
C20F	10-8-2014	E. Steffen	E14	Inner	-47.77	-73.69	283	7.06	-26.96	5.06
C20F	10-14-2014	Canal Martínez	E98b	Middle	-47.82	-73.84	298	7.50	-26.66	5.43
C20F	10-10-2014	Canal Martínez	E12cm	Inner	-47.79	-73.62	178	10.92	-26.66	4.53
C20F	10-11-2014	Pascua	E10	Glacier-fjord	-48.17	-73.35	295	6.31	-26.64	4.96
C20F	10-10-2014	Canal Troya	E11	Middle	-47.88	-73.74	334	8.32	-25.69	5.69
C20F	10-15-2014	Canal Martínez	E97	Middle	-47.78	-74.02	126	7.38	-25.38	8.62
C20F	10-4-2014	Canal Martínez	E5p	Outer	-47.89	-74.55	62	8.71	-22.37	8.41
C20F	10-15-2014	Golfo de Penas	E93	Outer	-47.37	-74.64	140	7.56	-21.44	8.40
C20F	10-15-2014	Canal Martínez	E03	Outer	-47.74	-74.76	270	8.35	-20.89	8.87
COPAS 2013	3-6-2013	Fiordo Jorge Montt	E2	Glacier-fjord	-48.29	-73.46	70	1.77	-29.36	-1.07
COPAS 2013	3-6-2013	Fiordo Jorge Montt	E3	Glacier-fjord	-48.19	-73.49	30	3.43	-29.17	-2.39
COPAS 2013	11-7-2013	Tortel	T3	Inner	-47.81	-73.54	20	4.94	-27.92	n.d.
COPAS 2013	11-7-2013	Tortel	T2	Inner	-47.81	-73.54	15	4.64	-26.87	1.15
COPAS 2013	11-8-2013	Fiordo Jorge Montt	E5B	Glacier-fjord	-48.19	-73.49	90	2.69	-26.82	2.37
COPAS 2013	3-6-2013	Fiordo Jorge Montt	E1	Glacier-fjord	-48.30	-73.46	77	2.14	-26.59	5.69
COPAS 2013	11-8-2013	Fiordo Jorge Montt	E4B	Glacier-fjord	-48.23	-73.47	70	3.21	-26.51	0.56
COPAS 2013	11-7-2013	Tortel	T1	Inner	-47.80	-73.53	10	4.32	-26.18	4.04
COPAS 2013	11-5-2013	Fiordo Jorge Montt	E1B	Glacier-fjord	-48.28	-73.44	46	4.54	-25.91	1.97
COPAS 2013	11-5-2013	Fiordo Jorge Montt	E3B	Glacier-fjord	-48.07	-73.54	30	3.83	-25.83	2.39
COPAS 2013	3-1-2013	Fiordo Jorge Montt	E6	Glacier-fjord	-48.16	-73.46	50	2.75	-25.44	1.72
COPAS 2013	3-8-2013	Fiordo Jorge Montt	E4	Glacier-fjord	-48.24	-73.47	80	3.37	-25.43	4.98
COPAS 2013	11-5-2013	Fiordo Jorge Montt	E2B	Glacier-fjord	-48.19	-73.51	35	4.03	-24.04	7.63
COPAS 2014	7-29-2014	Baker	E1Q	Inner	-47.79	-73.58	45	14.55	-26.27	4.55
COPAS 2014	10-19-2014	cercano río Pascua	E13	Glacier-fjord	-48.19	-73.34	124	7.05	-25.24	2.75
COPAS 2014	10-17-2014	Canal Martínez	E3	Middle	-47.81	-73.96	216	n.d.	-24.86	2.82
COPAS 2014	8-1-2014	Canal Martínez	E2Q	Middle	-47.78	-74.01	50	8.65	-24.23	2.64
COPAS 2014	10-15-2014	Tortel	E1	Inner	-47.79	-73.61	161	n.d.	-23.99	7.93
COPAS 2014	10-16-2014	Canal Martínez	Isla Irene	Middle	-47.81	-74.06	400	9.11	-23.41	7.10
COPAS 2014	10-13-2014	Canal Baker	E8	Middle	-47.95	-74.13	130	9.48	-23.00	7.05
COPAS 2014	10-16-2014	Canal Martínez	E4	Middle	-47.77	-74.23	261	8.54	-22.41	11.96
COPAS 2014	10-14-2014	Canal Martínez	E6e	Outer	-47.80	-74.60	180	8.43	-22.00	8.88
COPAS 2014	8-5-2014	Canal Baker	E3Q	Outer	-47.89	-74.67	40	8.48	-21.04	8.09
LOBOS 2016	1-29-2015	Baker	1	Inner	-47.79	-73.59	50	15.42	-28.39	1.42
LOBOS 2016	7-25-2014	Baker	1	Inner	-47.79	-73.59	50	11.06	-27.16	4.55
LOBOS 2016	3-25-2014	Baker	1	Inner	-47.79	-73.59	50	12.06	-26.88	n.d.
LOBOS 2016	1-26-2015	Merino Jarpa	5	Middle	-47.75	-74.22	50	11.08	-25.30	5.15
LOBOS 2016	7-26-2014	Merino Jarpa	5	Middle	-47.75	-74.22	50	7.94	-24.21	2.63
LOBOS 2016	3-27-2014	Merino Jarpa	5	Middle	-47.75	-74.22	50	13.89	-23.45	n.d.
LOBOS 2016	8-1-2014	Porcias	7	Outer	-47.89	-74.68	60	9.33	-21.81	6.59
LOBOS 2016	8-5-2014	Porcias	6	Outer	-47.88	-74.45	60	8.52	-21.77	8.09

Table S2.

Cruise	Date	Station	Environment	Latitude (°S)	Longitude (°W)	Depth (m)	N° Species	Abundance (Ind./1000 m^2)
CF25	Oct 2019	E46	Marine	-51.675	-75.194	149	21	1261
CF25	Oct 2019	F	Glacial	-50.712	-74.549	779	21	101
CF25	Oct 2019	E71	Glacial	-50.850	-73.922	254	24	167
CF25	Oct 2019	E50	Glacial	-52.006	-73.810	438	25	239
CF25	Oct 2019	OCE 3	Marine	-51.936	-75.518	103	40	2045
CF25	Oct 2019	E54	Hypoxic	-51.825	-72.831	179	15	242
CF23	Oct-Nov 2017	2	Marine	-47.619	-74.881	150	19	88
CF23	Oct-Nov 2017	3	Marine	-47.722	-74.776	240	18	292
CF23	Oct-Nov 2017	5	Marine	-47.900	-74.492	670	17	46
CF23	Oct-Nov 2017	10	Glacial	-48.151	-73.256	330	12	32
CF23	Oct-Nov 2017	17	Marine	-48.061	-74.638	1390	9	202
CF23	Oct-Nov 2017	18A	Glacial	-48.460	-73.986	270	10	29
CF23	Oct-Nov 2017	18C	Glacial	-48.523	-74.002	314	8	16
CF23	Oct-Nov 2017	19	Marine	-48.645	-74.375	314	19	133
CF23	Oct-Nov 2017	20	Marine	-48.892	-74.410	390	11	74
CF23	Oct-Nov 2017	21A	Glacial	-48.737	-74.261	164	7	23
CF23	Oct-Nov 2017	24	Marine	-49.362	-74.398	360	11	158
CF23	Oct-Nov 2017	25	Glacial	-49.562	-74.183	533	11	64
CF23	Oct-Nov 2017	28	Glacial	-49.281	-74.073	260	3	22
CF23	Oct-Nov 2017	30	Glacial	-49.527	-74.053	575	5	14
CF23	Oct-Nov 2017	33	Glacial	-49.922	-74.283	430	13	65
CF23	Oct-Nov 2017	35	Marine	-49.901	-74.387	800	12	34
CF23	Oct-Nov 2017	36	Glacial	-50.122	-74.237	452	10	24
CF23	Oct-Nov 2017	38	Glacial	-50.222	-74.143	400	9	40
CF23	Oct-Nov 2017	41	Marine	-50.348	-74.834	443	16	124
CF23	Oct-Nov 2017	42	Marine	-50.510	-74.977	570	17	244
CF23	Oct-Nov 2017	44	Marine	-51.208	-75.499	58	17	1535
CF23	Oct-Nov 2017	47	Marine	-51.590	-74.890	624	16	527
CF23	Oct-Nov 2017	48	Marine	-51.683	-74.347	770	4	8
CF23	Oct-Nov 2017	70	Glacial	-50.907	-74.251	240	22	180
CF23	Oct-Nov 2017	75	Glacial	-50.894	-73.832	152	8	124
CF23	Oct-Nov 2017	76	Glacial	-50.585	-74.248	390	13	136
CF23	Oct-Nov 2017	92	Marine	-47.403	-75.580	200	39	382
CF15	Oct-Nov 2009	36	Marine	-50.012	-74.237	250	5	39
CF15	Oct-Nov 2009	41	Marine	-50.348	-74.717	410	4	46
CF15	Oct-Nov 2009	57	Glacial	-51.817	-73.240	125	22	345
CF15	Oct-Nov 2009	58A	Glacial	-52.117	-73.247	280	24	963
CF15	Oct-Nov 2009	B	Glacial	-52.183	-72.537	26	1	2
CF15	Oct-Nov 2009	72	Glacial	-50.725	-73.803	354	9	21
CF15	Oct-Nov 2009	G	Marine	-50.793	-74.402	304	8	79
CF15	Oct-Nov 2009	43	Marine	-50.793	-75.213	254	5	7
CF15	Oct-Nov 2009	52	Glacial	-51.817	-72.558	25	3	5
CF15	Oct-Nov 2009	P	Hypoxic	-51.793	-72.850	170	4	29
CF15	Oct-Nov 2009	58	Glacial	-51.950	-73.283	140	22	1338
CF15	Oct-Nov 2009	60	Glacial	-52.065	-73.612	170	17	90
CF15	Oct-Nov 2009	49	Marine	-51.836	-74.131	620	9	138
CF7	Jul 2001	E1	Marine	-52.457	-68.583	70	12	60
CF7	Jul 2001	E2	Marine	-52.323	-69.203	40	9	19
CF7	Jul 2001	E3	Marine	-52.643	-69.775	24	14	25
CF7	Jul 2001	E4	Marine	-52.685	-70.170	41	9	17
CF7	Jul 2001	E5	Marine	-53.060	-70.547	88	19	144
CF7	Jul 2001	E6	Marine	-53.275	-70.687	177	22	129
CF7	Jul 2001	E56	Marine	-53.543	-69.912	270	5	21
CF7	Jul 2001	E55	Marine	-53.605	-70.267	50	3	55
CF7	Jul 2001	E8	Marine	-53.863	-72.533	260	6	22
CF7	Jul 2001	E9	Marine	-53.752	-71.998	313	17	49
CF7	Jul 2001	E10	Marine	-53.562	-72.477	404	16	81
CF7	Jul 2001	E15	Marine	-52.977	-73.810	604	5	8
CF7	Jul 2001	E14	Marine	-52.658	-74.808	66	6	36
CF7	Jul 2001	E12	Marine	-52.740	-74.927	90	10	54
CF7	Jul 2001	E16	Marine	-53.144	-73.078	411	6	22
CF3	Oct 1997	H8	Marine	-47.977	-74.220	723	3	2
CF3	Oct 1997	H5	Glacial	-48.005	-73.612	382	6	35
CF3	Oct 1997	H13	Marine	-48.705	-74.405	345	6	15
CF3	Oct 1997	H14	Marine	-46.898	-74.403	385	3	4
CF3	Oct 1997	H15	Marine	-49.040	-74.435	166	16	80
CF3	Oct 1997	H19	Glacial	-49.408	-74.098	349	5	6
CF3	Oct 1997	H22	Glacial	-49.535	-73.985	122	7	11
CF3	Oct 1997	H27	Marine	-49.888	-74.338	232	12	174
CF3	Oct 1997	H25	Glacial	-49.993	-74.062	298	7	9
CF3	Oct 1997	H32	Glacial	-50.225	-74.052	66	1	1
CF3	Oct 1997	H33S	Marine	-50.493	-75.042	75	34	204
CF3	Oct 1997	H35S	Glacial	-50.862	-74.098	117	12	31
CF3	Oct 1997	H39	Glacial	-50.505	-73.738	110	4	22
CF3	Oct 1997	H40	Glacial	-50.872	-73.847	148	5	11

Table S3.

No	Campaign	ID Sample	Location	Feeding modes	Faunal group	Taxa	$d^{13}C$	$d^{13}C_{Lipid\ corr}$	$d^{15}N$	C/N
1	LOBOS	SAMPLE E1	Inner	Carnivores	Decapoda	Libidoclaea granaria	-22.30	-22.0	7.97	3.65
2	LOBOS	SAMPLE E1	Inner	Carnivores	Decapoda	Libidoclaea granaria	-22.29	-22.0	7.95	3.65
3	LOBOS	SAMPLE E1	Inner	Carnivores	Decapoda	Libidoclaea granaria	-23.54	-23.2	6.93	3.71
4	LOBOS	SAMPLE E1	Inner	Carnivores	Decapoda	Libidoclaea granaria	-23.78	-23.5	6.54	3.68
5	LOBOS	SAMPLE E1	Inner	Carnivores	Decapoda	Peltarion spinulosum	-23.77	-23.5	6.71	3.71
6	LOBOS	SAMPLE E1	Inner	Carnivores	Decapoda	Peltarion spinulosum	-21.93	-21.4	8.37	4.01
7	LOBOS	SAMPLE E1	Inner	Carnivores	Decapoda	Peltarion spinulosum	-22.34	-22.0	7.98	3.70
8	LOBOS	SAMPLE E1	Inner	Carnivores	Decapoda	Peltarion spinulosum	-22.53	-22.2	7.92	3.71
9	LOBOS	SAMPLE FAUNA il 1a	Inner	Carnivores	Decapoda	Lithodes santolla	-21.35	-21.4	9.07	3.27
10	LOBOS	SAMPLE FAUNA il 1b	Inner	Carnivores	Decapoda	Lithodes santolla	-21.34	-21.4	9.14	3.30
11	LOBOS	SAMPLE FAUNA il 1c	Inner	Carnivores	Decapoda	Lithodes santolla	-21.53	-21.6	9.15	3.29
12	LOBOS	SAMPLE FAUNA il 30a	Inner	Carnivores	Decapoda	Libidoclaea granaria	-20.76	-21.0	8.74	3.12
13	LOBOS	SAMPLE FAUNA il 30b	Inner	Carnivores	Decapoda	Libidoclaea granaria	-20.73	-21.0	8.92	3.13
14	LOBOS	SAMPLE FAUNA il 31	Inner	Carnivores	Decapoda	Peltarion spinulosum	-23.24	-23.3	6.87	3.32
15	CF20	SAMPLE UMAG AM22	Inner	Deposit feeders	Echinodermata	Tripylaster philippii	-23.44	-21.5	8.91	8.21
16	CF20	SAMPLE UMAG AM23	Inner	Deposit feeders	Echinodermata	Tripylaster philippii	-24.02	-22.7	8.82	5.53
17	CF20	SAMPLE UMAG AM24	Inner	Deposit feeders	Echinodermata	Tripylaster philippii	-24.43	-23.1	8.82	5.62
18	LOBOS	SAMPLE E1	Inner	Deposit feeders	Gastropoda	Chilina chilensis	-23.33	-22.3	1.86	4.93
19	LOBOS	SAMPLE E1	Inner	Deposit feeders	Gastropoda	Chilina chilensis	-24.43	-23.2	2.48	5.29
20	LOBOS	SAMPLE E1	Inner	Deposit feeders	Gastropoda	Chilina chilensis	-27.17	-26.3	1.68	4.63
21	LOBOS	SAMPLE FAUNA il 4a	Inner	Herbivores	Gastropoda	Fusitriton cancellotus	-21.08	-20.7	9.02	3.80
22	LOBOS	SAMPLE FAUNA il 4b	Inner	Herbivores	Gastropoda	Fusitriton cancellotus	-21.33	-21.0	8.92	3.80
23	LOBOS	SAMPLE E1	Inner	Carnivores	Nemertina	Nemertinea sp	-22.01	-21.6	6.39	3.81
24	LOBOS	SAMPLE E1	Inner	Carnivores	Peracarida	Amphipoda unidentified	-20.17	-18.8	10.05	5.60
25	LOBOS	SAMPLE E1	Inner	Carnivores	Peracarida	Isopoda unidentified	-21.46	-19.9	7.98	6.32
26	LOBOS	SAMPLE E1	Inner	Carnivores	Pisces	Genypterus blacodes	-17.24	-17.4	14.61	3.20
27	LOBOS	SAMPLE E1	Inner	Carnivores	Pisces	Genypterus blacodes	-17.23	-17.4	14.59	3.19
28	LOBOS	SAMPLE E1	Inner	Carnivores	Pisces	Genypterus blacodes	-16.87	-17.0	14.79	3.19
29	LOBOS	SAMPLE FAUNA il 25a	Inner	Carnivores	Pisces	Schroederictis chilensis	-18.92	-19.3	12.36	3.03
30	LOBOS	SAMPLE FAUNA il 25b	Inner	Carnivores	Pisces	Schroederictis chilensis	-18.58	-19.1	12.49	2.90
31	LOBOS	SAMPLE FAUNA il 3	Inner	Carnivores	Polychaeta	Polychaeta Nephtyidae ind	-17.38	-17.0	11.78	3.74
32	LOBOS	SAMPLE E1	Inner	Deposit feeders	Polychaeta	Terebellidae unidentifed	-17.82	-15.5	5.27	11.16
33	LOBOS	SAMPLE E1	Inner	Deposit feeders	Polychaeta	Lumbrineridae unidentified	-24.23	-23.5	7.72	4.30
34	LOBOS	SAMPLE FAUNA il 2a	Middle	Carnivores	Decapoda	Lithodes santolla	-15.63	-15.7	12.81	3.29
35	LOBOS	SAMPLE FAUNA il 2b	Middle	Carnivores	Decapoda	Lithodes santolla	-15.37	-15.5	12.85	3.26
36	LOBOS	SAMPLE FAUNA il 22	Middle	Carnivores	Decapoda	Pagurus gaudauchi	-15.67	-15.8	13.21	3.27
37	LOBOS	SAMPLE FAUNA il 27	Middle	Carnivores	Decapoda	Parabunis granulosa	-17.67	-17.4	13.86	3.70
38	LOBOS	SAMPLE FAUNA il 28	Middle	Carnivores	Decapoda	Peltarium spinolosum	-16.93	-17.0	12.38	3.28
39	CF20	SAMPLE UMAG AM25	Middle	Deposit feeders	Decapoda	Munida subrugosa	-18.30	-17.9	10.19	3.77
40	CF20	SAMPLE UMAG AM26	Middle	Deposit feeders	Decapoda	Munida subrugosa	-19.47	-19.1	9.18	3.77
41	CF20	SAMPLE UMAG AM27	Middle	Deposit feeders	Decapoda	Munida subrugosa	-19.32	-18.8	9.61	4.00
42	CF20	SAMPLE UMAG AM34	Middle	Deposit feeders	Decapoda	Stereomastis suhimi	-18.41	-17.8	11.88	4.06
43	CF20	SAMPLE UMAG AM35	Middle	Deposit feeders	Decapoda	Stereomastis suhimi	-17.87	-17.3	12.94	4.13
44	CF20	SAMPLE UMAG AM36	Middle	Deposit feeders	Decapoda	Stereomastis suhimi	-18.84	-18.4	12.12	3.89
45	CF20	SAMPLE UMAG AM31	Middle	Carnivores	Echinodermata	Comasteria lurida	-16.24	-14.3	12.76	8.32
46	CF20	SAMPLE UMAG AM32	Middle	Carnivores	Echinodermata	Comasteria lurida	-15.27	-13.7	12.84	6.39
47	CF20	SAMPLE UMAG AM33	Middle	Carnivores	Echinodermata	Comasteria lurida	-15.44	-13.6	13.44	7.36
48	CF20	SAMPLE UMAG AM28	Middle	Deposit feeders	Echinodermata	Ophiuroidea unidentified	-12.26	-10.0	10.72	10.26
49	CF20	SAMPLE UMAG AM29	Middle	Deposit feeders	Echinodermata	Ophiuroidea unidentified	-12.15	-10.0	10.57	9.22
50	CF20	SAMPLE UMAG AM30	Middle	Deposit feeders	Echinodermata	Ophiuroidea unidentified	-11.86	-9.7	11.73	9.88
51	LOBOS	SAMPLE FAUNA il 29b	Middle	Herbivores	Gastropoda	Nacella magallanica	-14.94	-14.8	9.23	3.49
52	LOBOS	SAMPLE FAUNA il 29a	Middle	Herbivores	Gastropoda	Nacella magallanica	-14.79	-14.6	9.25	3.52
53	LOBOS	SAMPLE FAUNA il 15a	Middle	Carnivores	Pisces	Prolatilus jugularis	-15.59	-15.6	15.79	3.37
54	LOBOS	SAMPLE FAUNA il 15b	Middle	Carnivores	Pisces	Prolatilus jugularis	-15.47	-15.5	15.87	3.36
55	LOBOS	SAMPLE FAUNA il 15c	Middle	Carnivores	Pisces	Prolatilus jugularis	-15.41	-15.4	15.78	3.36
56	LOBOS	SAMPLE FAUNA il 16a	Middle	Carnivores	Pisces	Oncorhynchus mykiss	-18.31	-18.3	13.61	3.39
57	LOBOS	SAMPLE FAUNA il 16b	Middle	Carnivores	Pisces	Oncorhynchus mykiss	-18.34	-18.3	13.77	3.43
58	LOBOS	SAMPLE FAUNA il 17a	Middle	Carnivores	Pisces	Schroederictis chilensis	-15.84	-16.5	13.86	2.78
59	LOBOS	SAMPLE FAUNA il 18a	Middle	Carnivores	Pisces	Basilchys australis	-17.90	-18.0	13.39	3.28
60	LOBOS	SAMPLE FAUNA il 18b	Middle	Carnivores	Pisces	Basilchys australis	-18.31	-18.4	13.32	3.30
61	LOBOS	SAMPLE FAUNA il 20a	Middle	Carnivores	Pisces	Sebastes oculatus	-16.92	-16.5	14.71	3.88
62	LOBOS	SAMPLE FAUNA il 20b	Middle	Carnivores	Pisces	Sebastes oculatus	-16.89	-16.5	14.68	3.85
63	LOBOS	SAMPLE FAUNA il 19a	Middle	Carnivores	Pisces	Genypterus blacodes	-16.92	-17.2	13.35	3.13
64	LOBOS	SAMPLE FAUNA il 19b	Middle	Carnivores	Pisces	Genypterus blacodes	-16.85	-17.1	13.39	3.13
65	CF23	SAMPLE EQUIROGA B8	Outer	Suspension feeders	Bivalvia	Eunnucula grayi	-10.91	-8.3	10.28	14.67
66	CF23	SAMPLE EQUIROGA D10	Outer	Suspension feeders	Bivalvia	Cyclocardia velutina	-15.79	-15.7	10.65	3.51
67	CF23	SAMPLE EQUIROGA D11	Outer	Suspension feeders	Bivalvia	Eunnucula grayi	-18.32	-16.8	10.96	6.26
68	CF23	SAMPLE EQUIROGA F10	Outer	Suspension feeders	Bivalvia	Zygochlamys patagonica	-15.95	-15.8	11.25	3.47

Continuation Table S3.

No	Campaign	ID Sample	Location	Feeding modes	Faunal group	Taxa	$d^{13}C$	$d^{13}C_{Lipid\ corr}$	$d^{15}N$	C/N
69	LOBOS	SAMPLE FAUNA il 21a	Outer	Suspension feeders	Bivalvia	Mytilus chilensis	-19.17	-18.2	8.79	4.74
70	LOBOS	SAMPLE FAUNA il 21b	Outer	Suspension feeders	Bivalvia	Mytilus chilensis	-19.04	-18.1	8.85	4.75
71	LOBOS	SAMPLE FAUNA il 23	Outer	Suspension feeders	Bivalvia	Aulacomya atra	-19.47	-18.8	8.27	4.15
72	CF23	SAMPLE EQUIROGA C6	Outer	Suspension feeders	Brachiopoda	Brachiopoda unidentified	-17.62	-17.2	14.02	3.80
73	CF23	SAMPLE EQUIROGA C7	Outer	Suspension feeders	Brachiopoda	Limopsis marionensis	-15.53	-15.0	15.55	4.01
74	CF23	SAMPLE EQUIROGA G9	Outer	Suspension feeders	Cnidaria	Actinaria unidentified	-18.06	-17.1	9.61	4.75
75	LOBOS	SAMPLE FAUNA il 32	Outer	Carnivores	Decapoda	Metacarcinus edwarsii	-16.59	-16.3	14.10	3.65
76	LOBOS	SAMPLE FAUNA il 33a	Outer	Carnivores	Decapoda	Metacarcinus edwarsii	-15.87	-15.9	13.94	3.29
77	LOBOS	SAMPLE FAUNA il 33b	Outer	Carnivores	Decapoda	Metacarcinus edwarsii	-15.96	-16.0	13.84	3.29
78	CF23	SAMPLE EQUIROGA A3	Outer	Deposit feeders	Decapoda	Euphausidae unidentified	-16.13	-15.9	11.43	3.58
79	CF23	SAMPLE EQUIROGA C12	Outer	Deposit feeders	Decapoda	Sternaspis sp	-15.05	-15.7	10.25	2.81
80	CF23	SAMPLE EQUIROGA F11	Outer	Deposit feeders	Decapoda	Nauticaris magellanica	-17.08	-15.8	10.53	5.40
81	LOBOS	SAMPLE FAUNA il 34	Outer	Deposit feeders	Decapoda	Munida gregaria	-17.82	-17.4	11.31	3.81
82	CF20	SAMPLE UMAG AM38	Outer	Deposit feeders	Decapoda	Sternaspis scutata	-19.60	-18.6	12.01	4.84
83	CF20	SAMPLE UMAG AM39	Outer	Deposit feeders	Decapoda	Sternaspis scutata	-19.21	-18.2	11.85	4.76
84	CF20	SAMPLE UMAG AM40	Outer	Deposit feeders	Decapoda	Sternaspis scutata	-19.50	-18.4	12.19	4.99
85	CF23	SAMPLE EQUIROGA D2	Outer	Carnivores	Echinodermata	Asteroidea unidentified	-15.31	-14.4	14.56	4.54
86	CF23	SAMPLE EQUIROGA G5	Outer	Carnivores	Echinodermata	Asteroidea unidentified	-14.90	-13.5	13.36	5.92
87	CF23	SAMPLE EQUIROGA G6	Outer	Carnivores	Echinodermata	Glabraster antarctica	-15.59	-14.3	12.46	5.55
88	CF23	SAMPLE EQUIROGA G7	Outer	Carnivores	Echinodermata	Stichaster striatus	-14.93	-13.6	12.23	5.61
89	CF23	SAMPLE EQUIROGA G4	Outer	Deposit feeders	Echinodermata	Arbacia dufresnii	-15.24	-13.7	11.48	6.23
90	CF23	SAMPLE EQUIROGA A1	Outer	Deposit feeders	Echinodermata	Myxoderma qawashqari	-15.23	-14.0	16.38	5.45
91	CF23	SAMPLE EQUIROGA A2	Outer	Deposit feeders	Echinodermata	Cheiraster sp	-14.56	-12.2	12.71	11.22
92	CF23	SAMPLE EQUIROGA A4	Outer	Deposit feeders	Echinodermata	Ophiuroidea unidentified 1	-9.05	-7.0	12.70	8.67
93	CF23	SAMPLE EQUIROGA A7	Outer	Deposit feeders	Echinodermata	Tripylaster philippii	-10.82	-8.4	12.62	12.41
94	CF23	SAMPLE EQUIROGA B4	Outer	Deposit feeders	Echinodermata	Myxoderma qawashqari	-18.04	-17.7	11.71	3.81
95	CF23	SAMPLE EQUIROGA B9	Outer	Deposit feeders	Echinodermata	Tripylaster philippii	-11.15	-8.5	6.90	15.30
96	CF23	SAMPLE EQUIROGA B10	Outer	Deposit feeders	Echinodermata	Pseudoechinus magellanicus	-24.24	-22.7	9.21	6.15
97	CF23	SAMPLE EQUIROGA C1	Outer	Deposit feeders	Echinodermata	Myxoderma qawashqari	-16.30	-16.0	13.48	3.74
98	CF23	SAMPLE EQUIROGA D12	Outer	Deposit feeders	Echinodermata	Tripylaster philippii	-15.07	-15.7	11.84	2.87
99	CF23	SAMPLE EQUIROGA E1	Outer	Deposit feeders	Echinodermata	Schroedrichthys chilensis	-15.05	-15.3	13.61	3.17
100	CF23	SAMPLE EQUIROGA B3	Outer	Deposit feeders	Echinodermata	Gorgonocephalus chilensis	-20.27	-18.1	14.70	9.57
101	CF23	SAMPLE EQUIROGA F12	Outer	Deposit feeders	Echinodermata	Gorgonocephalus chilensis	-17.59	-16.3	10.89	5.58
102	CF23	SAMPLE EQUIROGA G1	Outer	Deposit feeders	Echinodermata	Gorgonocephalus chilensis	-17.75	-16.4	10.63	5.78
103	CF23	SAMPLE EQUIROGA G2	Outer	Deposit feeders	Echinodermata	Ophiomusa lymani	-17.30	-15.6	8.95	6.87
104	CF23	SAMPLE EQUIROGA G3	Outer	Deposit feeders	Echinodermata	Astrotoma agassizii	-15.45	-13.9	10.59	6.47
105	CF23	SAMPLE EQUIROGA G12	Outer	Deposit feeders	Echinodermata	Ophiuroidea unidentified 2	-13.63	-11.6	9.07	8.67
106	CF20	SAMPLE UMAG AM7	Outer	Deposit feeders	Echinodermata	Gorgonocephalus chilensis	-18.93	-18.2	14.04	4.38
107	CF20	SAMPLE UMAG AM8	Outer	Deposit feeders	Echinodermata	Gorgonocephalus chilensis	-18.79	-18.3	13.74	3.99
108	CF20	SAMPLE UMAG AM9	Outer	Deposit feeders	Echinodermata	Gorgonocephalus chilensis	-19.44	-18.6	13.71	4.59
109	CF20	SAMPLE UMAG AM10	Outer	Deposit feeders	Echinodermata	Ophiocten amitinum	-19.90	-19.1	9.88	4.47
110	CF20	SAMPLE UMAG AM11	Outer	Deposit feeders	Echinodermata	Ophiocten amitinum	-21.53	-20.1	8.78	6.01
111	CF20	SAMPLE UMAG AM12	Outer	Deposit feeders	Echinodermata	Ophiocten amitinum	-21.19	-19.9	9.57	5.45
112	CF20	SAMPLE UMAG AM13	Outer	Deposit feeders	Echinodermata	Ophiuroglypha lymani	-20.48	-19.3	10.07	5.10
113	CF20	SAMPLE UMAG AM14	Outer	Deposit feeders	Echinodermata	Ophiuroglypha lymani	-20.86	-19.7	9.56	5.21
114	CF20	SAMPLE UMAG AM15	Outer	Deposit feeders	Echinodermata	Ophiuroglypha lymani	-20.20	-19.2	10.78	4.79
115	CF23	SAMPLE EQUIROGA A10	Outer	Carnivores	Echiura	Echiura unidentified	-16.27	-15.3	12.76	4.80
116	CF23	SAMPLE EQUIROGA B7	Outer	Carnivores	Echiura	Echiura unidentified	-17.29	-17.2	8.85	3.51
117	CF23	SAMPLE EQUIROGA B6	Outer	Deposit feeders	Gastropoda	Dentalium majorinum	-17.14	-17.1	11.56	3.38
118	LOBOS	SAMPLE FAUNA il 24a	Outer	Herbivores	Gastropoda	Tegula atra	-16.62	-16.0	10.18	4.11
119	LOBOS	SAMPLE FAUNA il 24b	Outer	Herbivores	Gastropoda	Tegula atra	-16.73	-16.1	10.04	4.18
120	LOBOS	SAMPLE FAUNA il 26a	Outer	Herbivores	Gastropoda	Nacella magallanica	-13.04	-12.9	10.22	3.47
121	LOBOS	SAMPLE FAUNA il 26b	Outer	Herbivores	Gastropoda	Nacella magallanica	-12.92	-12.8	10.21	3.50
122	LOBOS	SAMPLE FAUNA il 26c	Outer	Herbivores	Gastropoda	Nacella magallanica	-12.92	-12.8	10.20	3.48
123	CF20	SAMPLE UMAG AM2	Outer	Suspension feeders	Hidrozoa	Corallium rubrum	-18.86	-19.0	13.41	3.27
124	CF20	SAMPLE UMAG AM3	Outer	Suspension feeders	Hidrozoa	Corallium rubrum	-19.65	-19.4	13.36	3.64
125	CF20	SAMPLE UMAG AM4	Outer	Suspension feeders	Hidrozoa	Hydrozoa unidentified 1	-20.73	-20.2	12.56	4.04
126	CF20	SAMPLE UMAG AM4B	Outer	Suspension feeders	Hidrozoa	Hydrozoa unidentified 2	-19.99	-19.7	13.31	3.72
127	CF20	SAMPLE UMAG AM5	Outer	Suspension feeders	Hidrozoa	Hydrozoa unidentified 1	-20.61	-20.0	13.61	4.17
128	CF20	SAMPLE UMAG AM6	Outer	Suspension feeders	Hidrozoa	Hydrozoa unidentified 1	-19.82	-19.4	13.56	3.91
129	CF20	SAMPLE UMAG AM16 VF2	Outer	Suspension feeders	Hidrozoa	Errina chilensis	-23.50	-22.0	9.10	6.15
130	CF20	SAMPLE UMAG AM17 VF2	Outer	Suspension feeders	Hidrozoa	Errina chilensis	-23.58	-22.5	4.88	4.95
131	CF20	SAMPLE UMAG AM18 VF2	Outer	Suspension feeders	Hidrozoa	Errina chilensis	-22.37	-21.5	7.01	4.52
132	CF20	SAMPLE UMAG AM19	Outer	Suspension feeders	Hidrozoa	Anthozoa unidentified	-19.22	-18.5	13.85	4.33
133	CF20	SAMPLE UMAG AM20	Outer	Suspension feeders	Hidrozoa	Anthozoa unidentified	-19.22	-18.6	14.09	4.20
134	CF20	SAMPLE UMAG AM21	Outer	Suspension feeders	Hidrozoa	Anthozoa unidentified	-19.24	-18.6	13.83	4.18
135	CF23	SAMPLE EQUIROGA A11	Outer	Carnivores	Nemertina	Nemertinea unidentified	-16.71	-16.4	16.93	3.75
136	CF23	SAMPLE EQUIROGA B12	Outer	Carnivores	Nemertina	Nemertinea unidentified	-14.23	-13.5	16.08	4.34
137	CF23	SAMPLE EQUIROGA B1	Outer	Deposit feeders	Peracarida	Mysidacea unidentified	-18.17	-17.6	10.62	4.05
138	CF23	SAMPLE EQUIROGA E6	Outer	Deposit feeders	Peracarida	Aega sp.	-15.53	-15.2	12.75	3.73

Continuation Table S3.

No	Campaign	ID Sample	Location	Feeding modes	Faunal group	Taxa	$\delta^{13}C$	$\delta^{13}C_{Lipid\ corr}$	$\delta^{15}N$	C/N
139	CF23	SAMPLE EQUIROGA A12	Outer	Carnivores	Pisces	Perciforme unidentified	-15.06	-13.8	12.72	5.52
140	CF23	SAMPLE EQUIROGA C4	Outer	Carnivores	Pisces	Perciforme unidentified	-15.14	-15.2	16.35	3.30
141	CF23	SAMPLE EQUIROGA C5	Outer	Carnivores	Pisces	Perciforme unidentified	-19.28	-18.4	10.73	4.60
142	CF23	SAMPLE EQUIROGA C10	Outer	Carnivores	Pisces	Macrouridae unidentified 1	-14.73	-15.0	15.13	3.13
143	CF23	SAMPLE EQUIROGA C11	Outer	Carnivores	Pisces	Macrouridae unidentified 1	-13.71	-13.5	15.18	3.59
144	CF23	SAMPLE EQUIROGA D3	Outer	Carnivores	Pisces	Mixinidae unidentified	-14.62	-14.5	13.75	3.46
145	CF23	SAMPLE EQUIROGA E2	Outer	Carnivores	Pisces	Salilota australis	-16.08	-16.2	13.30	3.30
146	CF23	SAMPLE EQUIROGA E3	Outer	Carnivores	Pisces	Salilota australis	-15.68	-15.7	13.68	3.33
147	CF23	SAMPLE EQUIROGA E4	Outer	Carnivores	Pisces	Hyppoglossina macrops	-15.84	-15.8	13.68	3.37
148	CF23	SAMPLE EQUIROGA E5	Outer	Carnivores	Pisces	Hyppoglossina macrops	-15.29	-14.3	11.29	4.71
149	CF23	SAMPLE EQUIROGA F1	Outer	Carnivores	Pisces	Macrouridae unidentifie 2	-14.65	-14.8	13.51	3.26
150	CF23	SAMPLE EQUIROGA F2	Outer	Carnivores	Pisces	Macrouridae unidentifie 2	-14.46	-14.6	13.75	3.23
151	CF23	SAMPLE EQUIROGA F3	Outer	Carnivores	Pisces	Macrouridae unidentifie 2	-14.95	-15.2	13.23	3.14
152	CF23	SAMPLE EQUIROGA F4	Outer	Carnivores	Pisces	Sebastes capensis	-14.28	-14.5	13.37	3.16
153	CF23	SAMPLE EQUIROGA F5	Outer	Carnivores	Pisces	Sebastes capensis	-14.36	-14.6	13.47	3.18
154	CF23	SAMPLE EQUIROGA F6	Outer	Carnivores	Pisces	Sebastes capensis	-15.01	-15.2	13.85	3.19
155	CF23	SAMPLE EQUIROGA F7	Outer	Carnivores	Pisces	Osteichthyes unidentified	-14.88	-15.1	13.53	3.18
156	CF23	SAMPLE EQUIROGA F8	Outer	Carnivores	Pisces	Osteichthyes unidentified	-15.03	-15.2	13.46	3.19
157	CF23	SAMPLE EQUIROGA F9	Outer	Carnivores	Pisces	Osteichthyes unidentified	-16.40	-16.4	8.11	3.38
158	CF23	SAMPLE EQUIROGA A6	Outer	Carnivores	Polychaeta	Lumbrineridae unidentified	-16.56	-16.1	13.64	3.93
159	CF23	SAMPLE EQUIROGA B2	Outer	Carnivores	Polychaeta	Polynoidea unidentified	-15.79	-15.6	13.90	3.58
160	CF23	SAMPLE EQUIROGA C3	Outer	Carnivores	Polychaeta	Polynoidae unidentified	-15.44	-15.5	14.81	3.29
161	CF23	SAMPLE EQUIROGA D4	Outer	Carnivores	Polychaeta	Leanira sp.	-16.86	-16.3	11.83	4.08
162	CF23	SAMPLE EQUIROGA D9	Outer	Carnivores	Polychaeta	Polychaeta unidentified	-15.04	-14.9	12.39	3.56
163	CF23	SAMPLE EQUIROGA E9	Outer	Carnivores	Polychaeta	Polynoidae unidentified	-15.91	-15.5	12.37	3.84
164	CF23	SAMPLE EQUIROGA E10	Outer	Carnivores	Polychaeta	Leanira sp.	-16.56	-16.1	9.65	3.87
165	CF23	SAMPLE EQUIROGA G8	Outer	Carnivores	Polychaeta	Polynoidae unidentified 2	-13.41	-13.5	13.55	3.28
166	CF23	SAMPLE EQUIROGA B5	Outer	Deposit feeders	Polychaeta	Ninoe leptognatha	-13.08	-11.4	9.35	6.78
167	CF23	SAMPLE EQUIROGA C2	Outer	Deposit feeders	Polychaeta	Lumbrineridae unidentified	-15.78	-15.8	12.81	3.39
168	CF23	SAMPLE EQUIROGA D5	Outer	Deposit feeders	Polychaeta	Hyalinoecia unidentified	-18.46	-17.2	12.36	5.42
169	CF23	SAMPLE EQUIROGA D7	Outer	Deposit feeders	Polychaeta	Maldane sarsi	-17.08	-16.6	10.12	3.95
170	CF23	SAMPLE EQUIROGA D8	Outer	Deposit feeders	Polychaeta	Lumbrineridae unidentified	-15.34	-15.1	13.67	3.61
171	CF23	SAMPLE EQUIROGA E7	Outer	Deposit feeders	Polychaeta	Lumbrineridae unidentified	-17.49	-16.6	11.65	4.56
172	CF23	SAMPLE EQUIROGA E11	Outer	Deposit feeders	Polychaeta	Hyalinoecia unidentified	-15.92	-15.4	12.80	3.96
173	CF23	SAMPLE EQUIROGA E12	Outer	Deposit feeders	Polychaeta	Maldane sarsi	-14.99	-15.1	12.87	3.25
174	CF20	SAMPLE UMAG AM42A	Outer	Deposit feeders	Polychaeta	Maldane sarsi	-18.36	-17.7	14.42	4.17
175	CF20	SAMPLE UMAG AM42B	Outer	Deposit feeders	Polychaeta	Maldane sarsi	-17.33	-17.4	12.46	3.34
176	CF20	SAMPLE UMAG AM43	Outer	Deposit feeders	Polychaeta	Maldane sarsi	-17.69	-17.6	12.43	3.47
177	CF20	SAMPLE UMAG AM44	Outer	Deposit feeders	Polychaeta	Maldane sarsi	-17.89	-17.2	13.84	4.24
178	CF20	SAMPLE UMAG AM44B	Outer	Deposit feeders	Polychaeta	Maldane sarsi	-17.66	-17.6	12.48	3.46
179	CF23	SAMPLE EQUIROGA A5	Outer	Suspension feeders	Polychaeta	Terebellidae unidentified	-21.19	-19.8	11.71	5.71
180	CF23	SAMPLE EQUIROGA B11	Outer	Suspension feeders	Polychaeta	Terebellidae unidentified	-21.91	-21.2	9.58	4.31
181	CF23	SAMPLE EQUIROGA D6	Outer	Suspension feeders	Polychaeta	Ampharetidae unidentified	-16.69	-15.9	12.58	4.48
182	CF23	SAMPLE EQUIROGA E8	Outer	Suspension feeders	Polychaeta	Ampharetidae unidentified	-16.47	-16.2	11.59	3.65
183	CF23	SAMPLE EQUIROGA G11	Outer	Suspension feeders	Polychaeta	Ampharetidae unidentified	-15.25	-14.8	9.14	3.90
184	CF23	SAMPLE EQUIROGA C8	Outer	Deposit feeders	Priapulida	Priapulida unidentified	-15.95	-15.4	15.18	4.00
185	CF23	SAMPLE EQUIROGA C9	Outer	Deposit feeders	Priapulida	Priapulida unidentified	-14.83	-15.1	15.13	3.15
186	CF23	SAMPLE EQUIROGA D1	Outer	Deposit feeders	Priapulida	Priapulida unidentified	-16.26	-14.7	13.85	6.27
187	CF23	SAMPLE EQUIROGA A8	Outer	Deposit feeders	Sipuncula	Sipunculidae unidentified	-13.98	-14.5	11.05	2.90
188	CF23	SAMPLE EQUIROGA A9	Outer	Deposit feeders	Sipuncula	Sipunculidae unidentified	-14.75	-14.8	14.18	3.33
189	CF23	SAMPLE EQUIROGA G10	Outer	Deposit feeders	Sipuncula	Sipunculidae unidentified	-14.72	-15.0	9.38	3.09

$\delta^{13}C_{Lipid\ corr}$: Logan et al., 2018.

Table S4.

No.	Group	Taxa	Abundance (ind m^{-2})	SD (ind m^{-2})	%
1	Polychaeta	Levinsenia antarctica	2589	3663	14.51
2	Polychaeta	Capitella sp	2565	3188	14.38
3	Polychaeta	Aphelochaeta cf marioni	2238	2871	12.55
4	Polychaeta	Aricidea antarctica	1584	2685	8.88
5	Polychaeta	Prionospio (Minuspio) chilensis	1310	1725	7.35
6	Mollusca	Thyasira patagonica	928	1013	5.20
7	Polychaeta	Aricidea strelzovi	675	1443	3.79
8	Polychaeta	Spiophanes soederstroemi	548	855	3.07
9	Polychaeta	Cossura sp	474	819	2.65
10	Polychaeta	Ophellidae sp A	438	752	2.45
11	Polychaeta	Aglaophamus peruana	313	365	1.75
12	Polychaeta	Lumbrineris cingulata	308	411	1.73
13	Polychaeta	Exogone sp	197	527	1.11
14	Polychaeta	Dorvilleidae sp A	188	270	1.05
15	Polychaeta	Lumbrineris magalhaensis	178	320	1.00
16	Polychaeta	Leanira quatrefagesi	163	109	0.92
17	Polychaeta	Aphelochaeta sp.	151	363	0.85
18	Mollusca	Yoldiella sp A	144	213	0.81
19	Crustacea	Diastylidae sp A	142	168	0.80
20	Nemertinea	Nemertinea unidentified A	139	135	0.78
21	Polychaeta	Ampharetidae sp A	132	180	0.74
22	Polychaeta	Ninoe falklandica	130	141	0.73
23	Polychaeta	Terebellides kerguelensis	123	172	0.69
24	Polychaeta	Terebellidae sp A	120	250	0.67
25	Polychaeta	Maldane sarsi	103	168	0.58
26	Polychaeta	Scoloplos sp	87	111	0.49
27	Mollusca	Bivalvia unidentified B	82	216	0.46
28	Mollusca	Yoldiella sp. C	72	140	0.40
29	Crustacea	Ostracoda sp B	67	149	0.38
30	Polychaeta	Paramphinome australis	63	103	0.35
31	Polychaeta	Glycera sp	58	148	0.32
32	Mollusca	Bivalvia unidentified A	55	122	0.31
33	Mollusca	Macoma sp	55	93	0.31
34	Echiura	Echiuridae sp A	53	100	0.30
35	Polychaeta	Nephtys sp A	53	122	0.30
36	Polychaeta	Syllidae sp A	53	120	0.30
37	Mollusca	Gastropoda unidentifed A	50	120	0.28
38	Polychaeta	Aglaophamus erectanoides	50	128	0.28
39	Crustacea	Heterophoxus sp	48	98	0.27
40	Nemertinea	Nemertinea unidentified B	48	74	0.27
41	Sipuncula	Sipuncula sp A	48	67	0.27
42	Mollusca	Aplacophora unidentified A	46	63	0.26
43	Polychaeta	Spiophanes sp.	43	95	0.24
44	Crustacea	Ostracoda sp A	41	67	0.23
45	Mollusca	Eunnucula sp	36	72	0.20
46	Crustacea	Gammaridae sp A	34	75	0.19
47	Polychaeta	Gyptis sp	34	53	0.19
48	Polychaeta	Ampharetidae sp B	31	88	0.18
49	Crustacea	Notatolana albinota	29	34	0.16
50	Mollusca	Scaphopoda unidentified	29	60	0.16
51	Polychaeta	Artacamella sp.	29	82	0.16
52	Polychaeta	Nereidae sp A	29	40	0.16
53	Polychaeta	Ophelia sp	26	75	0.15
54	Polychaeta	Phyllodocidae sp A	26	32	0.15
55	Polychaeta	Sphaeroridae sp A	26	52	0.15
56	Crustacea	Ampeliscidae sp A	22	40	0.12
57	Crustacea	Amphipoda C indet	22	40	0.12
58	Polychaeta	Aglaophamus heteroserrata	22	61	0.12
59	Polychaeta	Sabellidae sp A	22	26	0.12
60	Mollusca	Chaetoderma sp A	19	47	0.11
61	Crustacea	Amphipoda unidentified	17	22	0.09
62	Crustacea	Phoxocephalidae sp A	17	32	0.09
63	Mollusca	Nucula sp	17	48	0.09
64	Polychaeta	Aricidea albatrosae	17	48	0.09
65	Polychaeta	Melinna sp	17	35	0.09
66	Crustacea	Cumaceo sp.	14	41	0.08
67	Nemertinea	Nemertinea unidentified D	14	41	0.08
68	Polychaeta	Abyssoninoe abyssorum	14	27	0.08
69	Polychaeta	Hesionidae sp A	14	22	0.08
70	Polychaeta	Ophelina scaphingera	14	27	0.08
71	Crustacea	Tanaidáceo sp	12	34	0.07
72	Nemertinea	Nemertinea unidentified C	12	34	0.07
73	Polychaeta	Amage sp	12	27	0.07
74	Polychaeta	Aphroditidae sp A	12	27	0.07
75	Polychaeta	Goniadidae sp A	12	34	0.07
76	Polychaeta	Laonice cirrata	12	27	0.07
77	Polychaeta	Lysilla sp	12	34	0.07
78	Polychaeta	Mageloma sp A	12	34	0.07
79	Priapulida	Priapulida unidentified	12	14	0.07
80	Polychaeta	Aricidea finitima	10	27	0.05
81	Polychaeta	Dorvilleidae sp D	10	21	0.05
82	Polychaeta	Pilargiidae sp A	10	27	0.05
83	Brachiopoda	Brachiopoda sp B	7	20	0.04
84	Crustacea	Isopoda A indet	7	14	0.04
85	Crustacea	Oeciderotidae sp A	7	20	0.04
86	Mollusca	Lucinoma sp.	7	14	0.04
87	Polychaeta	Dorvilleidae sp B	7	20	0.04
88	Polychaeta	Euphionella lobulata	7	20	0.04
89	Polychaeta	Harmathoe sp	7	20	0.04
90	Polychaeta	Trichobranchidae sp A	7	20	0.04
91	Crustacea	Leuconidea sp A	5	14	0.03
92	Echinodermata	Ophiuroidea unidentified	5	9	0.03
93	Mollusca	Yoldiella sp B	5	14	0.03
94	Polychaeta	Ceratocephale sp	5	14	0.03
95	Polychaeta	Dorvilleidae sp C	5	14	0.03
96	Polychaeta	Eteone sp	5	14	0.03
97	Polychaeta	Nephtys sp B	5	14	0.03
98	Polychaeta	Onuphidae sp A	5	14	0.03
99	Polychaeta	Sabellidae indet B	5	14	0.03
100	Polychaeta	Sosanopsis sp.	5	14	0.03
101	Polychaeta	Trichobranchus glacialis	5	14	0.03
102	Brachiopoda	Brachiopoda sp.A	2	7	0.01
103	Crustacea	Decapoda sp A	2	7	0.01
104	Crustacea	Diastylidae sp B	2	7	0.01
105	Crustacea	Ostracoda sp C	2	7	0.01
106	Crustacea	Stilipedidae sp A	2	7	0.01
107	Crustacea	Pagurus sp	2	7	0.01
108	Echinodermata	Tripylaster philippii	2	7	0.01
109	Mollusca	Carditoidae sp A	2	7	0.01
110	Mollusca	Turridae sp A	2	7	0.01
111	Pantopoda	Anoplodactylus californicus	2	7	0.01
112	Polychaeta	Amaeana sp	2	7	0.01
113	Polychaeta	Antinoella antarctica	2	7	0.01
114	Polychaeta	Aricidea (Allia) suecica	2	7	0.01
115	Polychaeta	Cistenides ehlersi	2	7	0.01
116	Polychaeta	Nereidae sp B	2	7	0.01
117	Polychaeta	Polycirrus sp	2	7	0.01
118	Polychaeta	Sthenelais helenae	2	7	0.01
119	Sipuncula	Sipuncula sp B	2	7	0.01
120	Sipuncula	Sipuncula sp C	2	7	0.01

Table S5.

	Sum of sqrs	df	Mean square	F	p-value
Adj. mean:	125.571	2	62.7856	12.3	0.0001
Adj. error:	944.173	185	5.10364		
Adj. total:	1069.74	187			
Homogeneity (equality) of slopes:					
F :	11.39				
p-value	0.0001				

Table S6.

	Fjord	Corg (mg/g)	tCorg (%)	mCorg BR ($gC\ m^{-2}\ y^{-1}$)	tCorg BR ($gC\ m^{-2}\ y^{-1}$)
Arctic	Raunefjord	33.2	37.0	43	28
	Balsfjord	17.4	20.0	12	2
	Ullsfjord	17.8	17.0	9	1
	Hornsund	16.6	56.0	12	16
	Kongsfjord	13.2	33.0	20	8
	Rijpfjord	15.7	32.0	6	2
	Mean	19.0	32.5	17.0	9.5
Patagonian	Moraleda channel	18.2	11.3	11	1
	Jacaf fjord	31.1	13.7	20	3
	Ventisquero sound	24.1	51.2	23	25
	Puyuhuapi fjord	21.3	36.7	9	5
	Aysen fjord	17.4	41.5	6	4
	Costa Channel	18.2	35.5	9	5
	Quitralco fjord	17.7	33.9	12	6
	Cupquelan fjord	5.6	58.0	1	2
	Elefantes channel	4.0	50.6	1	1
	Martinez-Baker fjords	13.5	62.0	n.d.	n.d.
	Mean	17.1	39.4	10.3	5.9

Data source: Sepulveda et al., 2011; Włodarska –Kowalczuk et al., 2019a

Table S7.

Environment	Variable	Inner fjord		Middle fjord		Outer fjord	
		Mean	SD	Mean	SD	Mean	SD
Water column	POC (µg L^{-1})	212	16	252	20	268	11
	N/P	11.8		17.1		17.4	
	Silicate $Si(OH)_4$ (µM)	17.9	18.2	15.1	9.2	6.8	5.8
Sediment	$\delta^{13}C$ (‰)	-26.6	1.1	-24.4	1.7	-21.6	0.5
	$\delta^{15}N$ (‰)	3.7	2.0	5.5	2.8	8.1	0.7
	C/N	10.1	4.1	9.5	2.1	8.4	0.5
Macrofauna	Diversity (H')	2.7	0.1	3.3	0.1	2.7	0.1
	N° Species	32	5	37	9	44	13
	Abundances (ind m^{-2})	3756	1473	1474	875	4309	829
	Biomass (gC m^{-2})	4.0	1.4	3.9	1.0	4.6	1.3
	$\delta^{13}C$ (‰)	-21.1	2.3	-16.0	2.5	-16.2	2.7
	$\delta^{15}N$ (‰)	8.5	3.1	12.7	1.9	12.1	2.2
	C/N	4.3	1.7	4.5	2.1	4.6	2.0

N/P= NO_3+NO_2/PO_4 (µM)

Quiroga et al., 2016; Cari et al., 2020

Chapter 4

Synthesis of the results and general discussion

What is the influence of allochthonous organic matter in the faunal composition and trophic structure in epibenthic communities in the *Puyuhuapi* fjord?

The benthic community of the *Puyuhuapi* fjord exhibited high values of species richness, associated to several types of environments (hard and soft bottoms). In hard bottom environments 95 taxa/species were registered; the majority of them were suspension feeding organisms (58%) and were mostly characterized by Porifera, Cnidaria, Echinodermata and Mollusca. In soft bottom environments, 49 taxa/species were recorded, where infauna represented by polychaetes and large decapods appear to be dominant as depositivores (51%). Our results showed that environmental variables define the composition and distribution of benthic communities, but the depth, substrate inclination and lateral coupling (land-ocean influence) were key factors in determining the benthic epifauna distribution, and the contributions of organic sources.

It is known that in the *Puyuhuapi* fjord the suspended material may influence the distribution of benthic epifauna, favoring greater diversity in areas with low sedimentation rates (Betti et al., 2017; 2021). Suspension feeding (SS) organisms were frequently observed in the stations located in the mouth and outer end of the *Puyuhuapi* fjord, and were one of most diverse group. SS organisms included sponges, octocorals, bivalves, polychaetes, bryozoans, holothuroids, and ascidians. The middle section of the *Puyuhuapi* fjord presents, higher concentration of suspension material which was in turn associated to a high abundance of sessile organisms, mainly encrusting porifera and octocorales (SS), as well as mollusks, polychaetes and echinoderms. In terms of feeding modes, we found diverse feeding guilds. In soft bottom environments, we found a well-defined spatial gradient related to the input of allochthonous organic matter (e.g. terrestrial organic matter, urban discharges, salmon farming waste). Stations close to allochthonous organic matter sources exhibited functional

traits (i.e. high abundances of families of opportunistic polychaetes) associated with organic pollution. Moreover, the complexity of substrates (mud, sand, detritus, rock) and allochthonous food sources, determined functional traits, and feeding modes for many depositivores and suspension feeding organisms in the study area.

In the *Puyuhuapi* fjord, allochthonous terrestrial sources make an important tOM contribution to benthic feeders. Salmon farming waste also contributes with organic carbon and nitrogen sources as dissolved and particulate organic matter in the water column and sediments (Buschmann et al., 2009; Niklitschek et al., 2013; Quiñones et al., 2019). It is known that benthic communities are highly sensitive to organic enrichment, modifying the community structure and impacting the environment (e.g., Brown et al., 1987; Gowen and Bradbury, 1987; Weston, 1990; Wildish and Pohle, 2005; Keeley et al., 2019; Sanchis et al., 2021).

Our results of the isotopic composition of carbon and nitrogen in food sources showed low $\delta^{13}C$ values in SOM (-28‰) and SPOM (-25‰), indicating an important contribution of tOM to the food web. These isotopic values – consistent with previous studies in the Patagonian fjords (Sepulveda et al., 2011; Silva et al., 2011; González et al., 2019; Cari et al., 2020) – indicate that rivers and runoffs are a high OM subsidy (e.g., emergent vascular terrestrial woody plants and freshwater phytoplankton) to the benthic ecosystem (66–96%) particularly in the continental fjord environments such as the *Puyuhuapi* fjord (Silva et al., 2011; Sepulveda et al., 2011). In general, potential food source contributions to consumers indicate that macroalgae predominated over other sources (SOM and SPOM), exhibiting a major contribution in grazers (GR) and carnivores (CA). The SS exhibited the highest proportions of SPOM and SOM as food source compared others trophic groups.

Suspension feeders were subdivided in three subgroups given the differences in $\delta^{13}C$ and $\delta^{15}N$ values; SS1 subgroup (porifera and cnidaria) exhibited depleted carbon and enriched nitrogen signals and presented the highest isotopic niche width (SEAc= 10.22). This group was followed by grazers (SEAc = 6.32), carnivores (SEAc = 3.92), and the SS2 and SS3 groups (SEAc = 2.71 and SEAc = 2.21, respectively). All functional groups showed overlap, with higher areas of niche overlap for SS and GR groups. The bivalve mollusks (SS2) showed isotopic signals probably related to phytoplankton and/or resuspended detritus of macroalgae

(Bearham et al., 2020). Benthic grazers showed a predominantly macroalgae diet, and a mixture of food sources associated with a major isotopic niche width, and higher $\delta^{13}C$ and lowest $\delta^{15}N$ values compared to SS and CA groups. CA presented an isotopic niche associated with high $\delta^{15}N$ values, within the range reported for predators in other areas of the Chilean Patagonia.

Our results also indicate low $\delta^{13}C$ values in *C. chilensis* are associated with significant tOM contributions, the higher $\delta^{15}N$ values appear to be related to nitrogen compounds derived from salmon farms; however, here holobiont-microbiome interactions may also be involved (Kahn et al., 2018). High $\delta^{15}N$ values - similar to those registered in large-predator species such as asteroidea, crustaceans, and gastropods -were also found in an encrusting octocoral (*I. comauensis*; 15.98 ± 0.82‰). In fact, suspension-feeders such as sponges, cnidarians and tunicates, which relied on naturally $\delta^{15}N$-depleted sources, have been registered as $\delta^{15}N$-enriched near fish farms (Lojen et al., 2005; Dolenec et al., 2007; Wai et al., 2011; Callier et al., 2013). Suspension feeders such as *C. chilensis* and *I. comauensis* may feed on dissolved OM and therefore assimilate an important fraction of dissolved nitrogen (Anthony, 1999; Pita et al., 2018; Rix et al., 2020; Maldonado et al., 2021). Moreover, the SS1 group also exhibited trophic niche overlap with the carnivore group, suggesting that it may be involved in nitrogen compound assimilation from salmon fish waste.

This study showed that isotopic niche width of benthic species in the *Puyuhuapi* fjord was highly variable, probably in response to food supply derived from marine and terrestrial OM. These results suggest that *C. chilensis* and *I. comauensis* may have assimilated an important fraction of dissolved nitrogen probably derived from salmon farming waste, exhibiting enriched values of $\delta^{15}N$. However, the contribution of nitrogen to the food web, shaped a benthic community with higher trophic redundancy, with different isotopic signals for the trophic group of suspension feeders, and showed sponges and cold-water corals (SS1) $\delta^{15}N$-enriched.

In general, the OM isotopic composition showed environmental gradients, reflecting the dynamics between riverine organic matter and horizontal and vertical transport, characteristic of highly stratified estuarine environment with marked suspended particulate material longitudinal gradients. In our study area, glaciomarine and glaciofluvial environmental stress

affected nutrient reservoirs and organic matter (OM) availability negatively and hence produced changes in benthic diversity and trophic structure throughout the fjords. Glaciomarine environments showed lower species richness where small-bodied polychaetes and opportunistic species dominated. This faunal structure correlates to the distribution of tOM in sediments, which gradually decrease towards the outer fjord. Furthermore, there is a C/N ratio decoupling in benthic fauna, suggesting preferential organic carbon use under limited nitrogen in fjord sediments. In fact, organismal C/N ratios varied among fjords, ranging with mean values 4.26 ±0.19 for the *Reloncavi* fjord, 4.16 ±0.19 for the Interior Sea of *Chiloé*, and 4.52 ±0.08 for the *Puyuhuapi* fjord. While these values indicate that OM originates from primary production and terrestrial plants and debris, the *Martinez-Baker* Fjord C/N ratio (4.28 ±0.29) – with its low level of nitrogen – appears to be associated with terrestrial OM and glacier activity (Rebolledo et al., 2019). The higher C/N ratio in the *Puyuhuapi* fjord seems to be related with allochthonous nitrogen inputs; indeed, salmon farming activities and nitrogen inputs are closely associated with aquaculture.

Benthic communities in Chilean fjords are intimately connected to oceanographic conditions and suspended sediments, particularly in the inner fjords. Since the Chilean fjord region has proven to be one of the most sensitive to climate change (Iriarte et al., 2018), consistent records of its benthic marine biodiversity patterns, isotopic compositions, and stoichiometric imbalances will be useful ecological indicators at a global level. They will additionally act as a bellwether for possible environmental impacts in the coming decades since expected shifts in rainfall regimes and air temperature in the region (Garreaud et al., 2013). Such changes in freshwater inputs and nutrients will certainly affect primary productivity negatively, with apparent consequences to the biodiversity and functioning of Patagonian benthic ecosystems.

At a local scale, in the *Puyuhuapi* fjord, different environmental and anthropogenic forces act as drivers of change in the structure and composition of the benthic communities, such as allochthonous sources of OM coming mainly from glaciofluvial discharges with high contributions of tOM and salmon farming waste. This high OM contribution in addition to anoxic conditions reported in the water column and low ventilation of the deep micro-basin

waters (Schneider et al. 2014) put the *Puyuhuapi* fjord in an extremely vulnerable position, considering that the load carrying, and adaptation capacity of this system is utterly unknown.

Since benthic communities play an important role in the OM cycling in fjord ecosystems, we suggest that suspension feeders such as *C. chilensis* and *I. comauensis* in the *Puyuhuapi* fjord, may be used as ecological indicators to evaluate stoichiometry imbalances. Environmental monitoring programs may make use of them to provide essential baseline information in identifying and trace different sources of organic pollution in fjord environments. In this sense, the spatial and functional information of benthic communities are a fundamental input for a marine spatial planning approach (Ehler and Douvere, 2009; O'Learly et al., 2021), which must urgently be incorporated into the planning of the coastal zone of the Chilean Patagonia.

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